(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71751 A1

(51) International Patent Classification[7]: C12Q 1/68, C12P 21/06, 19/34, A01N 43/04, 37/18, A61K 38/00, 38/28, C07H 21/02, 21/04

(21) International Application Number: PCT/US00/13327

(22) International Filing Date: 16 May 2000 (16.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/135,423 21 May 1999 (21.05.1999) US
60/174,700 6 January 2000 (06.01.2000) US

(71) Applicant: MYRIAD GENETICS, INC. [US/US]; 320 Wakara Way, Salt Lake City, UT 84108 (US).

(72) Inventors: MCGRAIL, Maura; 2125 Texas St., Salt Lake City, UT 84109 (US). RUSSELL, Deanna, L.; 2731 South Hartford St., Salt Lake City, UT 84106 (US). SHATTUCK, Donna, M.; 1874 Texas St., Salt Lake City, UT 84108 (US).

(74) Agents: IHNEN, Jeffrey, L. et al.; Rothwell, Figg, Ernst & Manbeck, Suite 701 East, 555 13th Street N.W., Columbia Square, Washington, DC 20004 (US).

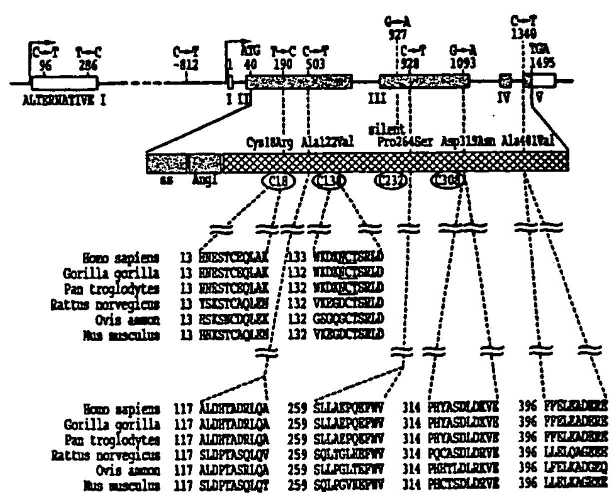
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

[Continued on next page]

(54) Title: DIABETES GENE



(57) Abstract: The present invention relates generally to the field of human genetics. Specifically, the present invention relates to methods and materials used to isolate and detect human diabetes mellitus predisposing gene, specifically the angiotensinogen (AGT) gene, some mutant alleles of which cause susceptibility to insulin-dependent diabetes mellitus (IDDM). More specifically, the invention relates to germline mutations in the *AGT* gene and their use in the diagnosis of predisposition to diabetes. The invention also relates to the prophylaxis and/or therapy of diabetes associated with a mutation in the *AGT* gene. The invention further relates to the screening of drugs for diabetes therapy. Finally, the invention relates to the screening of the *AGT* gene for mutations, which are useful for diagnosing the predisposition to diabetes.

WO 00/71751 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

DIABETES GENE

BACKGROUND OF THE INVENTION

The present invention relates generally to the field of human genetics. Specifically, the present invention relates to methods and materials used to isolate and detect human diabetes mellitus predisposing gene, specifically the angiotensinogen (AGT) gene, some mutant alleles of which cause susceptibility to insulin-dependent diabetes melitus (IDDM). More specifically, the invention relates to germline mutations in the *AGT* gene and their use in the diagnosis of predisposition to IDDM. The invention also relates to the prophylaxis and/or therapy of diabetes associated with a mutation in the *AGT* gene. The invention further relates to the screening of drugs for diabetes therapy. Finally, the invention relates to the screening of the *AGT* gene for mutations, which are useful for diagnosing the predisposition to diabetes.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference, and for convenience, are referenced by author and date in the following text and respectively grouped in the appended List of References.

Diabetes mellitus is among the most common of all metabolic disorders, affecting up to 11% of the population by age 70. Type I diabetes (insulin dependent diabetes mellitus or IDDM) represents about 5 to 10% of this group and is the result of a progressive autoimmune destruction of the pancreatic β-cells with subsequent insulin deficiency. IDDM is characterized by a partial or complete inability to produce insulin. Patients with IDDM would die without daily insulin injections to control their disease.

Few advancements in resolving the pathogenesis of diabetes were made until the mid-1970s when evidence began to accumulate to suggest that IDDM had an autoimmune etiopathogenesis. It is now generally accepted that IDDM results from the chronic autoimmune destruction of the insulin producing pancreatic β-cells. Lymphocytes and other inflammatory cells have been observed within the islets of Langerhans in newly diagnosed IDDM patients and have been found preferentially in regenerating islets composed of β-cells rather than those of

other cell types. This active immunological process is associated with a variety of autoantibodies to β-cells cytoplasmic and membrane antigens, insulin, and insulin receptors. Although the general mechanism by which IDDM occurs is known, IDDM becomes clinically evident only after the vast majority of pancreatic β-cells have been irrevocably destroyed and the individual becomes dependent upon exogenous insulin.

In both humans and diabetes-prone non-obese diabetic (NOD) mice, genes mapping to the major histocompatibility complex have been associated with susceptibility to IDDM and shown to be very important in the disease process (Todd, 1990). Studies of NOD mice have mapped at least 12 other susceptibility genes to specific chromosomal locations (Prochazaka et al., 1987; Todd et al., 1991; De Gouyon et al., 1993; Morahan et al., 1994; Serreze et al., 1994; Cornall et al., 1991; Garchon et al., 1991). In humans, markers near the insulin/insulin-like growth factor loci have also been associated with IDDM (Bell et al., 1984).

Epidemiological and molecular genetic studies indicate that only 40% of the genetic susceptibility is due to alleles of the MHC class II genes, suggesting additional non-MHC genes are involved (Todd et al., 1988; Buzzetti. et al., 1998). Genome-wide searches and analyses of specific genes have identified more at least 17 loci that contribute to the disease (Buzzetti et al., 1998; Todd, 1995; Concannon et al., 1998; Hashimoto et al., 1994; Davies et al., 1994; Mein et al., 1998; Verge et al., 1998). Significant evidence for linkage was reported for a ~7cM region on chromosome 1q42-43 (Concannon et al., 1998). This region of chromosome 1 contains the angiotensinogen (*AGT*) gene.

Non-insulin dependent diabetes mellitus (NIDDM) is one of the most common inherited diseases in man with an estimated prevalence in Caucasian populations of 8-10% (Harris et al., 1987). It is estimated to affect more than 100 million people worldwide (King and Zimmer, 1988; Harris et al., 1992). Clinically, NIDDM is a heterogenous disorder characterized by chronic hyperglycemia leading to progressive micro- and macrovascular lesions in the cardiovascular, renal and visual systems as well as diabetic neuropathy. The causes of the fasting hyperglycemia and/or glucose intolerance associated with this form of diabetes are not well understood. Unfortunately, the disease is associated with early morbidity and mortality. Subtypes of the disease can be identified based at least to some degree on the time of onset of the symptoms. The principal type of NIDDM occurs at a later time of onset, typically at midlife. Early-onset NIDDM or maturity-onset diabetes of the young (MODY) shares many features with the more common form(s) of NIDDM but onset occurs in early life.

Although most forms of NIDDM do not exhibit simple Mendelian inheritance, the contribution of heredity is well recognized (Rotter et al., 1990). The genetic basis of a few rare monogenic syndromes of NIDDM have been elucidated, but together, these syndromes account for a very small minority of cases (Taylor et al., 1992; Froguel et al., 1993; Steiner et al., 1990; Kadowaki et al., 1994). It is likely that the common forms of NIDDM are complex and heterogenous, and result when a pool of mutant genes, each of which contributes modestly and in a subtle way, interact with each other and with environmental, aging and behavioral influences to lead to the expression of the disease. This pool of genes may vary between populations and among individuals within a population, despite the illusion of a clinically homogenous phenotype.

Certain loci have been linked to rare early-onset forms of Type II diabetes that is associated with chronic hyperglycemia and monogenic inheritance (i.e. maturity onset diabetes of the young (MODY) loci) (Bell et al., 1991; Froguel et al., 1992; Hattersley et al., 1992; Vaxillaire et al., 1995). The defects in the glucokinase (GCK) gene on human chromosome 7 have been found to be responsible for the relatively rare MODY2 phenotype. (Froguel et al., 1992).

The genes responsible for MODY1 and MODY3 have been identified to be transcription factors hepatocyte nuclear factor 4 (HNF4) and hepatocyte nuclear factor 1 HNF1, repsectively. (Yamagata et al., 1996a; Yamagata et al. 1996b). The diabetes gene HNF4/MODY1 regulates expression of the AGT gene (Yanai et al., 1999). Mutations in HNF4/MODY1 have been demonstrated to cause predisposition to maturity onset diabetes of the young.(MODY) a genetically heterogenouse monogenic form of non-insulin-dependent diabetes mellitus (NIDDM) (Yamagata et al., 1996b; Lindner et al., 1997).

Another locus has been identified for a rare early-onset form with mitochondrial inheritance (Van den Ouwenland et al., 1992). In addition, Harris et al. (1996) identified a locus of NIDDM1 on chromosome 2 that appears to play a role in Mexican American diabetes. Further, Mahtani et al. (1996) report evidence of the existence of a gene on human chromosome 12, NIDDM2, that causes NIDDM associated with low insulin secretion. The paper suggests that NIDDM2 and MODY3 represent different alleles of the same gene with severe mutations causing MODY3 and milder mutations giving rise to later-onset NIDDM characterized by low insulin secretion.

In view of the heterogeneity of genes associated with IDDM or NIDDM, it is desired to identify additional genes associated with IDDM or NIDDM for diagnostic and therapeutic purposes.

SUMMARY OF THE INVENTION

Tight linkage between DNA polymorphisms in and near the angiotensinogen (AGT) gene on chromosome 1 and diabetes in particular families was observed (Concannon, 1999). This invention provides the first evidence implicating specific mutations in the AGT gene with susceptibility to IDDM.

Thus, in one aspect of the invention, novel nucleic acids containing additional genomic sequences are provided. These nucleic acids are set forth in SEQ ID NOs:1-5 and 8.

In a second aspect of the invention, a method for detecting a susceptibility in an individual to insulin-dependent diabetes mellitus is provided. Thus, the present invention provides methods for determining whether a subject is at risk for developing diabetes due to a mutation in the AGT gene. This method relies on the fact that mutations in the AGT have been correlated by the inventors with the disease. It will be understood by those of skill in the art, given the disclosure of the invention that such mutations are associated with a susceptability to diabetes, that a variety of methods may be utilized to detect mutations in the AGT gene, including the mutations disclosed herein, which are associated with a susceptability to IDDM.

The method can include detecting, in a tissue of the subject, the presence or absence of a polymorphism of the AGT gene. The detection of a polymorphism in the AGT gene may include ascertaining the existence of at least one of: a deletion of one or more nucleotides; an addition of one or more nucleotides, a substitution of one or more nucleotides; a gross chromosomal rearrangement; an alteration in the level of a messenger RNA transcript; the presence of a non-wild type splicing pattern of a messenger RNA transcript; a non-wild type level of an AGT protein; and/or an aberrant level of an AGT protein.

For example, detecting the polymorphism can include (i) providing a probe/primer comprised of an oligonucleotide which hybridizes to a sense or antisense sequence of an AGT gene or naturally occurring mutants thereof, or 5' or 3' flanking sequences naturally associated with an AGT gene; (ii) contacting the probe/primer to an appropriate nucleic acid containing sample; and (iii) detecting, by hybridization of the probe/primer to the nucleic acid, the presence or absence of the polymorphism; e.g. wherein detecting the polymorphism comprises utilizing the probe/primer to determine the nucleotide sequence of an AGT gene and, optionally, of the

flanking nucleic acid sequences. For instance, the primer can be employed in a polymerase chain reaction (PCR), in a ligase chain reaction (LCR) or other amplification reactions known to a skilled artisan. In alternate embodiments, the level of an AGT protein is detected in an immunoassay using an antibody which is specifically immunoreactive with the AGT protein.

In a third aspect of the invention, compounds that are agonists or antagonists of a normal (functional) AGT bioactivity and their use in preventing or treating diabetes are provided. For example, to ameliorate disease symptoms involving insufficient expression of an AGT gene and/or inadequate amount of functional AGT bioactivity in a subject, a gene therapeutic (comprising a gene encoding a functional AGT protein) or a protein therapeutic (comprising a functional AGT protein or fragment thereof) can be administered to the subject. Alternatively, agonists or antagonists of AGT function (wild-type or mutant) or an AGT receptor or a receptor for fragments of AGT can be administered.

In a fourth aspect of the invention, compounds that are antagonists of a disease causing AGT bioactivity and their use in preventing or treating diabetes are provided. For example, to ameliorate disease symptoms involving expression of a mutant AGT gene or aberrant expression of a normal AGT gene in a subject, a therapeutically effective amount of an antisense, ribozyme or triple helix molecule to reduce or prevent gene expression may be administered to the subject. Alternatively, to ameliorate disease symptoms involving the regulation via an AGT protein or AGT protein fragments of an upstream or downstream element in an AGT mediated biochemical pathway (e.g. signal transduction), a therapeutically effective amount of an agonist or antagonist compound (e.g. small molecule, peptide, peptidomimetic, protein or antibody) that can prevent normal binding of the wildtype AGT protein, can induce a therapeutic effect.

In another aspect of the invention, assays, e.g., for screening test compounds to identify antagonists (e.g. inhibitors), or alternatively, agonists (e.g. potentiators), of an interaction between an AGT protein and, for example, a protein or nucleic acid that binds to the AGT protein or fragments of AGT are provided. An exemplary method includes the steps of (i) combining an AGT polypeptide or bioactive fragments thereof, an AGT target molecule (such as an AGT ligand or nucleic acid), and a test compound, e.g., under conditions wherein, but for the test compound, the AGT protein and AGT target molecule are able to interact; and (ii) detecting the formation of a complex which includes the AGT protein and the target molecule either by directly quantitating the complex or by measuring inductive effects of the AGT protein or fragments of AGT protein. A statistically significant change, such as a decrease, in the interaction of the AGT and AGT target molecule in the presence of a test compound (relative to

what is detected in the absence of the test compound) is indicative of a modulation (e.g., inhibition or potentiation of the interaction between the AGT protein or fragments of the AGT protein and the target molecule).

In a further aspect of the present invention concerns methods for modulating the transcription of certain genes in a cell by modulating AGT bioactivity, (e.g., by potentiating or disrupting an AGT bioactivity). In general, whether carried out *in vivo*, *in vitro*, or *in situ*, the method comprises treating the cell with an effective amount of an AGT therapeutic (agonist or antagonist of an AGT bioactivity) so as to alter, relative to the cell in the absence of treatment, the level of transcription of certain genes. Accordingly, the method can be carried out with AGT therapeutics such as peptide and peptidomimetics or other molecules identified in the above-referenced drug screens which agonize or antagonize the effects of a AGT bioactivity (e.g. transcription) of a gene which is regulated by an AGT protein. Other AGT therapeutics include antisense constructs for inhibiting expression of AGT proteins, and dominant negative mutants of AGT proteins which competitively inhibit interactions between ligands (e.g. proteins) and nucleic acids upstream and downstream of the wild-type AGT protein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C show a physical map of the *AGT* region. Fig. 1A: Microsatellite markers used in genotyping. The STS *WI18298* used to screen the BAC library lies on BAC 1-30. Fig. 1B: BACs containing the *AGT* gene. Fig. 1C: structure of *AGT* and alignment on BAC contig.

Figures 2A-2E show haplotypes and segregation of *AGT* mutations in multiplex type 1 diabetes families. Shared haplotypes are in shaded boxes. Fig. 2A: Cys18Arg. Fig. 2B: C(96)T in alternative exon 1. Fig. 2C: T(286)C in alternative exon 1. Fig. 2D: Pro264Ser linked to a silent polymorphism is present on the haplotype with the asterisk in family 30. Fig. 2E: C(-811)T is on the same haplotype in family 30 as Pro264Ser.

Figures 3A-3C show rare polymorphisms in *AGT* and expression of alternatively spliced transcripts. Fig. 1A: Genomic organization of *AGT* and position of rare polymorphisms found in diabetes mellitus families. Approximate positions of promoters for the alternative and published transcripts are indicated by large arrows. The positions and types of rare polymorphisms identified in multiplex diabetes families are indicated above the exons. Within the coding exons the numbers refer to the base pair position in the cDNA. The polymorphism at –811 is relative to the published transcription start site in exon I (Gaillard et al., 1989). A schematic of the AGT polypeptide is shown below. The positions of the amino acid substitutions caused by the

polymorphisms are indicated relative to the start of the mature peptide. Coding region of AGT (black box); signal sequence(ss) (gray box); Ang I precursor peptide to the octapeptide Ang II (black box); mature peptide (black box and hatched box). Greater than 100kb of genomic DNA (dashed line). All Cys positions of AGT indicated in a circle. Fig. 3B: Alignment of angiotensinogen from human, gorilla, chimpanzee, rat, sheep, and mouse is shown. Cys18 in bold; Asn137-Cys138-Thr139 underlined. Fig. 3C: Alignment of additional missense mutations: Ala122, Pro264, Asp319, and Ala401 shown in bold.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based in part on the discovery of polymorphisms in the *AGT* gene which are linked to insulin-dependent diabetes mellitus. Based on this finding the invention provides therapeutic methods, compositions and diagnostics for diabetes based on AGT.

The cDNA for the AGT gene and the protein sequence are set forth in GenBank accession numbers K02215 and AH002594. The latter accession shows some genomic sequences (about 40-60 nucleotides) surrounding the 5 exons of the AGT gene. The AGT gene comprises 5 exons which are shown in SEQ ID NOS:1-5, along with genomic intron or untranslated sequences. SEQ ID NOs: 1-5 contain additional genomic sequences not previously disclosed. The AGT gene encodes a protein which comprises a 33 amino acid signal peptide and a 452 amino acid mature peptide. The coding sequence for AGT compiled from the exons in SEQ ID NOs:1-5 is shown in SEQ ID NO:6. The corresponding amino acid sequence for AGT is set forth in SEQ ID NO:7. The sequence of alternate exon 1 along with surrounding genomic intron or untranslated sequences is shown in SEQ ID NO:8.

The present invention relates to AGT agonists and antagonists and their use in treating diabetes. For example, (i) nucleic acid molecules encoding functional AGT protein; (ii) nucleic acids that are effective antisense, ribozyme and triplex antagonists of nucleic acids encoding functional AGT protein; (iii) functional AGT proteins or peptides; (iv) anti-AGT antibodies; (v) drugs affecting wild-type or mutant AGT function or AGT interaction with an AGT receptor and preparations of such compositions are disclosed herein. In addition, the invention provides drug discovery assays for identifying additional agents that agonize or antagonize the biological function of AGT protein (e.g. by altering the interaction of AGT molecules with either downstream or upstream elements in the biochemical (e.g. signal transduction) pathway).

Moreover, the present invention provides assays for diagnosing whether a subject has or has a predisposition towards developing diabetes mellitus.

Proof that any particular gene located within the genetically defined interval is a disease susceptability locus is obtained by finding sequences in DNA or RNA extracted from affected kindred members which create abnormal gene products or abnormal levels of gene product. Such disease susceptibility alleles will co-segregate with the disease in large kindreds. They will also be present at a much higher frequency in non-kindred individuals with the disease than in individuals in the general population. In identifying a disease susceptability locus, the key is to find polymorphisms or mutations which are serious enough to cause obvious disruption to the normal function of the gene product. These mutations can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice versa, or other mutations which would affect secondary, tertiary or quaternary protein structure. Small deletions or base pair substitutions could also significantly alter protein expression by changing the level of transcription, splice pattern, mRNA stability, or translation efficiency of the gene transcript. Silent mutations or those resulting in conservative amino acid substitutions would not generally be expected to disrupt protein function. Causal mutations can also be found in the promoter of the gene. These mutations would interfere with the binding of regulatory factors and in this way alter transcription of the gene and therefore change the function of the gene.

In one aspect, the invention features probes and primers for use in a prognostic or diagnostic assay. For instance, the present invention also provides a probe/primer comprising a substantially purified oligonucleotide, which oligonucleotide comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least approximately 12, preferably 25, more preferably 40, 50 or 75 consecutive nucleotides of sense or anti-sense sequence of AGT, including 5' and/or 3' untranslated regions. In preferred embodiments, the probe further comprises a label group attached thereto and able to be detected, e.g. the label group is selected from amongst radioisotopes, fluorescent compounds, enzymes, and enzyme co-factors.

In a further aspect, the present invention features methods for determining whether a subject is at risk for developing diabetes mellitus. According to the diagnostic and prognostic

methods of the present invention, alteration of the wild-type AGT locus is detected. "Alteration of a wild-type gene" encompasses all forms of mutations including deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gene or of only a portion of the gene. Point mutations may result in stop codons, frameshift mutations or amino acid substitutions. Point mutations or deletions in the promoter can change transcription and thereby alter the gene function. Somatic mutations are those which occur only in certain tissues and are not inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. The finding of AGT germline mutations thus provides diagnostic information. An AGT allele which is not deleted (e.g., found on the sister chromosome to a chromosome carrying an AGT deletion) can be screened for other mutations, such as insertions, small deletions, and point mutations. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, or in intron regions or at intron/exon junctions.

Useful diagnostic techniques include, but are not limited to fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis and PCR-SSCP, as discussed in detail further below. Also useful is the recently developed technique of DNA microchip technology. In addition to the techniques described herein, similar and other useful techniques are also described in U.S. Patent Nos. 5,837,492 and 5,800,998, each incorporated herein by reference.

Predisposition to disease can be ascertained by testing any tissue of a human for mutations of the AGT gene. For example, a person who has inherited a germline AGT mutation would be prone to develop IDDM. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the AGT gene. Alteration of a wild-type AGT allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. For a gene as large as AGT, manual sequencing is very labor-intensive, but under optimal conditions, mutations in the coding sequence of a gene are rarely missed. Another approach is the single-stranded conformation polymorphism assay (SSCA) (Orita *et al.*,

1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCA makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCA gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield *et al.*, 1991), heteroduplex analysis (HA) (White *et al.*, 1992) and chemical mismatch cleavage (CMC) (Grompe *et al.*, 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation.

Detection of point mutations may be accomplished by molecular cloning of the AGT allele(s) and sequencing the allele(s) using techniques well known in the art. Alternatively, the gene sequences can be amplified directly from a genomic DNA preparation from the tissue, using known techniques. The DNA sequence of the amplified sequences can then be determined.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single-stranded conformation analysis (SSCA) (Orita *et al.*, 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell *et al.*, 1990; Sheffield *et al.*, 1989); 3) RNase protection assays (Finkelstein *et al.*, 1990; Kinszler *et al.*, 1991); 4) allele-specific oligonucleotides (ASOs) (Conner *et al.*, 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Rano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular AGT mutation. If the particular AGT mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton *et al.*, 1989. Insertions and deletions of genes can also

be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the AGT mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCA, DGGE and RNase protection assay), a new electrophoretic band appears. SSCA detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of tumor samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type AGT gene coding sequence. The riboprobe and either mRNA or DNA isolated from the tumor tissue are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the AGT mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the AGT mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton *et al.*, 1988; Shenk *et al.*, 1975; Novack *et al.*, 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR before hybridization. Changes in DNA of the AGT gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the AGT gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the AGT gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length (although shorter and longer oligomers are also usable as well recognized by those of skill in the art), corresponding to a portion of the AGT gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the AGT gene. Hybridization of allele-specific probes with amplified AGT sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates the presence of the same mutation in the tumor tissue as in the allele-specific probe.

The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can determine the presence of mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of many, even thousands, of probes at once and can tremendously increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996; Lipshutz et al., 1995. This method has already been used to screen people for mutations in the breast cancer gene BRCA1 (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Nature Genetics, 1996). Also see Fodor (1997).

The most definitive test for mutations in a candidate locus is to directly compare genomic AGT sequences from disease patients with those from a control population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from disease patients falling outside the coding region of AGT can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the AGT gene. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in disease patients as compared to control individuals.

Alteration of AGT mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished or increased mRNA expression indicates an alteration of the wild-type AGT gene. Alteration of wild-type AGT genes can also be detected by screening for alteration of wild-type AGT protein. For example, monoclonal antibodies immunoreactive with AGT can be used to screen a tissue. Lack of cognate antigen would indicate an AGT mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant AGT gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered AGT protein can be used to detect alteration of wild-type AGT genes. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect AGT biochemical function. Finding a mutant AGT gene product indicates alteration of a wild-type AGT gene.

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular AGT allele using PCR. The pairs of single-stranded DNA primers can be annealed to sequences within or surrounding the AGT gene on chromosome 1 in order to prime amplifying DNA synthesis of the AGT gene itself. A complete set of these primers allows synthesis of all of the nucleotides of the AGT gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele-specific primers can also be used. Such primers anneal only to particular AGT mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their 5' ends. Thus, all nucleotides of the primers are derived from AGT sequences or sequences adjacent to AGT, except for the few nucleotides

necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Given the known sequences of the AGT exons and the 5' alternate exon, the design of particular primers is well within the skill of the art. Suitable primers for mutation screening are also described herein.

The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the AGT gene or mRNA using other techniques.

It has been discovered that individuals with the wild-type AGT gene do not have IDDM which results from the AGT allele. However, mutations which interfere with the function of the AGT protein are involved in the susceptability to IDDM as shown herein. Thus, the presence of an altered (or a mutant) AGT gene which produces a protein having a loss of function, or altered function, directly correlates to an increased risk of disease. In order to detect an AGT gene mutation, a biological sample is prepared and analyzed for a difference between the sequence of the AGT allele being analyzed and the sequence of the wild-type AGT allele. Mutant AGT alleles can be initially identified by any of the techniques described above. The mutant alleles are then sequenced to identify the specific mutation of the particular mutant allele. Alternatively, mutant AGT alleles can be initially identified by identifying mutant (altered) AGT proteins, using conventional techniques. The mutant alleles are then sequenced to identify the specific mutation for each allele. The mutations, especially those which lead to an altered function of the AGT protein, are then used for the diagnostic methods of the present invention.

The present invention employs definitions commonly used in the art with specific reference to the gene described in the present application. Such definitions can be found in U.S. Patent Nos. 5,837,492 and 5,800,998, each incorporated herein by reference. Such definitions are employed herein unless the context indicates otherwise.

Methods of Use: Nucleic Acid Diagnosis and Diagnostic Kits

In order to detect the presence of an *AGT* allele predisposing an individual to diabetes, a biological sample such as blood is prepared and analyzed for the presence or absence of predisposing alleles of *AGT*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be

performed by diagnostic laboratories, or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis. Diagnositic or prognostic tests can be performed as described herein or using well known techniques, such as described in U.S. Patent No. 5,800,998, incorporated herein by reference.

Initially, the screening method involves amplification of the relevant *AGT* sequences. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based screening strategies can detect target sequences with a high level of sensitivity.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

When the probes are used to detect the presence of the target sequences (for example, in screening for diabetes susceptibility), the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence; e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of human chromosome 1. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in,

for example, Maniatis *et al.*, 1982 and Sambrook *et al.*, 1989. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadraplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these variations are reviewed in, e.g., Matthews and Kricka, 1988; Landegren *et al.*, 1988; Mittlin, 1989; U.S. Patent 4,868,105, and in EPO Publication No. 225,807.

As noted above, non-PCR based screening assays are also contemplated in this invention. This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a 10^3-10^6 increase in sensitivity. For an example relating to the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes see Jablonski *et al.*, 1986.

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding *AGT*. Allele specific probes are also contemplated within the scope of this example and exemplary allele specific probes include probes encompassing the predisposing or potentially predisposing mutations summarized in herein.

In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. For methods for labeling

nucleic acid probes according to this embodiment see Martin *et al.*, 1990. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. For methods for labeling nucleic acid probes and their use in biotin-avidin based assays see Rigby *et al.*, 1977 and Nguyen *et al.*, 1992.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of detecting *AGT*. Thus, in one example to detect the presence of *AGT* in a cell sample, more than one probe complementary to *AGT* is employed and in particular the number of different probes is alternatively 2, 3, or 5 different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *AGT* gene sequence in a patient, more than one probe complementary to *AGT* is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in *AGT*. In this embodiment, any number of probes can be used, and will preferably include probes corresponding to the major gene mutations identified as predisposing an individual to diabetes. Some candidate probes contemplated within the scope of the invention include probes that include the allele-specific mutations identified herein and those that have the *AGT* regions corresponding to SEQ ID NOs:1-5 and 8 both 5' and 3' to the mutation site.

<u>Methods of Use: Peptide Diagnosis and Diagnostic Kits</u>

Susceptibility to daibetes can also be detected on the basis of the alteration of wild-type AGT polypeptide. Peptide diagnositic or prognostic tests can be performed as described herein or using well known techniques, such as described in U.S. Patent No. 5,800,998, incorporated herein by reference. For example, such alterations can be determined by sequence analysis in accordance with conventional techniques. More preferably, antibodies (polyclonal or monoclonal) are used to detect differences in, or the absence of, AGT peptides. The antibodies may be prepared in accordance with conventional techniques. Other techniques for raising and purifying antibodies are well known in the art and any such techniques may be chosen to achieve the preparations claimed in this invention. In a preferred embodiment of the invention, antibodies will immunoprecipitate AGT proteins or fragments of the AGT protein from solution as well as react with AGT peptides on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect AGT proteins and protein fragments in paraffin or frozen tissue sections, using immunocytochemical techniques.

Preferred embodiments relating to methods for detecting AGT or its mutations include enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David *et al.* in U.S. Patent Nos. 4,376,110 and 4,486,530, hereby incorporated by reference.

Methods of Use: Drug Screening

This invention is particularly useful for screening compounds by using a wild-type or mutant AGT polypeptide or binding fragment thereof in any of a variety of drug screening techniques. Drug screening can be performed as described herein or using well known techniques, such as described in U.S. Patent Nos. 5,800,998 and 5,891,628, each incorporated herein by reference.

The AGT polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, or borne on a cell surface. One method of drug screening utilizes eucaryotic or procaryotic host cells which are stably transformed with recombinant polynucleotides expressing the polypeptide or fragment, preferably in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, for the formation of complexes between an AGT polypeptide or fragment and the agent being tested, or examine the degree to which the formation of a complex between an AGT polypeptide or fragment and a known ligand, e.g. AGT receptor (AT1), is interfered with by the agent being tested.

Thus, the present invention provides methods of screening for drugs comprising contacting such an agent with an AGT polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the AGT polypeptide or fragment, or (ii) for the presence of a complex between the AGT polypeptide or fragment and a ligand, by methods well known in the art. In such competitive binding assays the AGT polypeptide or fragment is typically labeled. Free AGT polypeptide or fragment is separated from that present in a protein:protein complex, and the amount of free (i.e., uncomplexed) label is a measure of the binding of the agent being tested to AGT or its interference with AGT:ligand binding, respectively.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the AGT polypeptides and is described in detail in Geysen, PCT published application WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate,

such as plastic pins or some other surface. The peptide test compounds are reacted with AGT polypeptides and washed. Bound AGT polypeptides are then detected by methods well known in the art.

Purified AGT can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to the polypeptide can be used to capture antibodies to immobilize the AGT polypeptide on the solid phase.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of specifically binding the AGT polypeptide compete with a test compound for binding to the AGT polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants of the AGT polypeptide.

A further technique for drug screening involves the use of host eukaryotic cell lines or cells (such as described above) which express a wild-type or mutant *AGT* gene and as a consequence of expression of wild type or mutant AGT demonstrate a specific phenotype.. The phenotype of the cells is examined to determine if the compound is capable of modulating the phenotype and thereby AGT function.

Briefly, a method of screening for a substance which modulates activity of a polypeptide may include contacting one or more test substances with the polypeptide in a suitable reaction medium, testing the activity of the treated polypeptide and comparing that activity with the activity of the polypeptide in comparable reaction medium untreated with the test substance or substances. A difference in activity between the treated and untreated polypeptides is indicative of a modulating effect of the relevant test substance or substances.

Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with the polypeptide, e.g., in a yeast two-hybrid system (e.g., Bartel et al., 1993; Fields and Song, 1989; Chevray and Nathans, 1992; Lee et al., 1995). This system may be used as a coarse screen prior to testing a substance for actual ability to modulate activity of the polypeptide. Alternatively, the screen could be used to screen test substances for binding to an AGT specific binding partner, or to find mimetics of an AGT polypeptide.

Methods of Use: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide

in vivo. See, e.g., Hodgson, 1991. Rational drug design can be performed as described herein or using well known techniques, such as described in U.S. Patent Nos. 5,800,998 and 5,891,628, each incorporated herein by reference.

In one approach, one first determines the three-dimensional structure of a protein of interest (e.g., AGT polypeptide or fragments of the AGT polypeptide) or, for example, of the AGT-receptor or ligand complex, by x-ray crystallography, by computer modeling or most typically, by a combination of approaches. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. An example of rational drug design is the development of HIV protease inhibitors (Erickson *et al.*, 1990). In addition, peptides (e.g., AGT polypeptide or fragments thereof) are analyzed by an alanine scan (Wells, 1991). In this technique, an amino acid residue is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is analyzed in this manner to determine the important regions of the peptide.

It is also possible to isolate a target-specific antibody, selected by a functional assay, and then to solve its crystal structure. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore. Thus, one may design drugs which have, e.g., improved AGT polypeptide activity or stability or which act as inhibitors, agonists, antagonists, etc. of AGT polypeptide activity.

Following identification of a substance which modulates or affects polypeptide activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e., manufacture or formulation, or a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

Thus, the present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of polypeptide activity, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a method comprising administration of such a composition comprising such a substance, a method comprising administration of such a composition to a patient, e.g., for treatment or prophylaxis of diabetes, use of such a substance in the manufacture

of a composition for administration, e.g., for treatment or prophylaxis of diabetes, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

A substance identified as a modulator of polypeptide function may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a mimetic or mimic of the substance (particularly if a peptide) may be designed for pharmaceutical use.

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., pure peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large numbers of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, e.g., by substituting each residue in turn. Alanine scans of peptide are commonly used to refine such peptide motifs. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties, e.g., stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g., spectroscopic techniques, x-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modeling process.

In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted onto it

can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. Alternatively, where the mimetic is peptide-based, further stability can be achieved by cyclizing the peptide, increasing its rigidity. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Methods of Use: Nucleic Acid Based Therapies

According to the present invention, a method is also provided of supplying wild-type AGT function to a cell which carries mutant AGT alleles. The wild-type AGT gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. If a gene fragment is introduced and expressed in a cell carrying a mutant AGT allele, the gene fragment should encode a part of the AGT protein which is required for normal physiological processes of the cell. More preferred is the situation where the wild-type AGT gene or a part thereof is introduced into the mutant cell in such a way that it recombines with the endogenous mutant AGT gene present in the cell. Such recombination requires a double recombination event which results in the correction of the AGT gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate coprecipitation and viral transduction are known in the art, and the choice of method is within the competence of the routineer. See also U.S. Patent Nos. 5,800,998 and 5,891,628, each incorporated by reference herein.

Among the compounds which may exhibit anti-diabetes activity are antisense, ribozyme, and triple helix molecules. Such molecules may be designed to reduce or inhibit mutant AGT activity. Techniques for the production and use of such molecules are well known to those of skill in the art, such as described herein or in U.S. Patent No. 5,800,998, incorporated herein by reference.

Antisense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. With respect to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between the -10 and +10 regions of the AGT nucleotide sequence of interest, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by a endonucleolytic cleavage. The composition of ribozyme molecules must include one or more sequences complementary to the target AGT mRNA, preferably the mutant AGT mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see U.S. Pat. No. 5,093,246, which is incorporated by reference herein in its entirety. As such, within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of RNA sequences encoding AGT, preferably mutant AGT proteins.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequence: GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features, such as secondary structure, that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Nucleic acid molecules to be used in triplex helix formation should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC.sup.+ triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molrcules may be chosen that are purine-rich, for example, contain a stretch of guanidine residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with one strand of a

duplex first and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

It is possible that the antisense, ribozyme, and/or triple helix molecules described herein may reduce or inhibit the translation of mRNA produced by both normal and mutant AGT alleles. In order to ensure that substantial normal levels of AGT activity are maintained in the cell, nucleic acid molecules that encode and express AGT polypeptides exhibiting normal AGT activity may be introduced into cells which do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments. Such sequences may be introduced via gene therapy methods. Alternatively, it may be preferable to coadminister normal AGT protein into the cell or tissue in order to maintain the requisite level of cellular or tissue AGT activity.

Antisense RNA and DNA molecules, ribozyme molecules and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various well-known modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

Gene therapy would be carried out according to generally accepted methods, for example, as described in further detail in U.S. Patent Nos. 5,837,492 and 5,800,998 and references cited therein, all incorporated by reference herein. Expression vectors in the context of gene therapy are meant to include those constructs containing sequences sufficient to express a polynucleotide that has been cloned therein. In viral expression vectors, the construct contains viral sequences sufficient to support packaging of the construct. If the polynucleotide encodes an antisense polynucleotide or a ribozyme, expression will produce the antisense polynucleotide or ribozyme. Thus in this context, expression does not require that a protein product be

synthesized. In addition to the polynucleotide cloned into the expression vector, the vector also contains a promoter functional in eukaryotic cells. The cloned polynucleotide sequence is under control of this promoter. Suitable eukaryotic promoters include those described above. The expression vector may also include sequences, such as selectable markers and other sequences conventionally used.

Methods of Use: Peptide Therapy

Peptides which have AGT activity can be supplied to cells which carry mutant or missing AGT alleles. Peptide therapy is performed as described herein or using well known techniques, such as described in U.S. Patent Nos. 5,800,998 and 5,891,628, each incorporated herein by reference.

Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, AGT polypeptide can be extracted from AGT-producing mammalian cells. In addition, the techniques of synthetic chemistry can be employed to synthesize AGT protein. Any of such techniques can provide the preparation of the present invention which comprises the AGT protein. Preparation is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or *in vitro*.

Active AGT molecules can be introduced into cells by microinjection or by use of liposomes, for example. Alternatively, some active molecules may be taken up by cells, actively or by diffusion. Extracellular application of the AGT gene product may be sufficient to affect the development and or progression of diabetes.. Supply of molecules with AGT activity should lead to partial reversal of thediabetic phenotype. Other molecules with AGT activity (for example, peptides, drugs or organic compounds) may also be used to effect such a reversal. Modified polypeptides having substantially similar function are also used for peptide therapy.

Alternatively, antibodies that are both specific for mutant AGT gene product and interfere with its activity may be used. Such antibodies may be generated using standard techniques described herein or using conventional techniques, such as described in U.S. Patent Nos. 5,837,492; 5,800,998 and 5,891,628, against the proteins themselves or against peptides corresponding to the binding domains of the proteins. Such antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, F(ab').sub.2 fragments, single chain antibodies, chimeric antibodies, humanized antibodies etc.

Methods of Use: Agonists or Antagonists for Diabetes Prevention or Treatment

Mutations in angiotensinogen predisposing to diabetes suggest that the protein or peptide fragments of the protein have roles in the development or continued functioning of the beta islet

cells of the pancreas. It is known from the literature that mutation of Cys18 leads to a reduction of the cleavage of angiotensinogen by renin (Rosa et al., 1998). However, there are a series of experiments which would be necessary in order to address angiotensinogen's role in the disease and to identify points for pharmacological intervention.

The only known peptide derived by cleavage of angiotensinogen with renin is angiotensin 1. Angiotensin 1 (AI) is then subsequently cleaved by angiotensin converting enzyme (ACE) to angiotensin 2 (AII) which is, itself, metabolized to angiotensin 3 (AIII) and smaller peptides. Two human receptors for angiotensin 2, AT1 and AT2, are known in the literature, but it has been reported that other receptors for these active metabolites exist (for example, Kozlowski et al., 1993), and can be molecularly cloned. These receptors may have a biological distribution that includes the pancreas and/or be selective for one or more of the angiotensin 1 metabolites. Such receptors represent good targets for pharmacological intervention in diabetes. Equally, agonists, for example AI, AII, AIII or synthetic derivatives such as those described by Samanen et al. (1991), or antagonists of the known angiotensin receptors (e.g., losartan) represent drugs for use in the treatment or prevention of diabetes. After the initial cleavage by renin a large polypetide remains. The breakdown of this polypeptide into other active fragments can be envisioned. Agonist and antagonists of the receptors for these fragments as well as the proteases responsible for there formation also represent drugs for the treatment or prevention of diabetes.

Methods of Use: Identification of Functional Peptides Derived From AGT

Nothing, in current literature, is known of the metabolic fate of the remainder of angiotensinogen once angiotensin 1 is cleaved off by renin. It is possible that this protein has a direct biological function or be cleaved to bio-active peptides. The existence of such peptides can be confirmed by using a panel of polyclonal antibodies which bind to angiotensingen and use these antibodies to look for immuno-reactive peptide fragments in human or other animal sera. Once such peptides are identified, their cognate receptors can be cloned using standard molecular biology approaches. Another approach would be to screen for receptor partners for random fragments of angiotensinogen using the yeast two hybrid system or other techniques to discover protein/protein interactions. The peptides, identified by any approach, may, themselves, have utility as drugs for the treatment of diabetes or their receptors may represent points for pharmacological intervention in diabetes. In addition the inhibitors or activators of the protease (s) responsible for the cleavage of the full length AGT protein represnet another opportunity to develop drugs to treat or prevent diabetes.

Methods of Use: Transformed Hosts; Transgenic/Knockout Animals and Models

Similarly, cells and animals which carry a mutant *AGT* allele can be used as model systems to study and test for substances which have potential as therapeutic agents. These may be isolated from individuals with *AGT* mutations, either somatic or germline. Alternatively, the cell line can be engineered to carry the mutation in the *AGT* allele, as described above. After a test substance is applied to the cells, the phenotype of the cell is determined. Any trait of the transformed cells can be assessed using techniques well known in the art. Transformed hosts, transgenic/knockout animals and models are prepared and used as described herein or using well known techniques, such as described in U.S. Patent Nos. 5,800,998 and 5,891,628, each incorporated herein by reference.

Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant *AGT* alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous *AGT* gene(s) of the animals may be disrupted by insertion or deletion mutation or other genetic alterations using conventional techniques (Capecchi, 1989; Valancius and Smithies, 1991; Hasty *et al.*, 1991; Shinkai *et al.*, 1992; Mombaerts *et al.*, 1992; Philpott *et al.*, 1992; Snouwaert *et al.*, 1992; Donehower *et al.*, 1992) to produce knockout or transplacement animals. A transplacement is similar to a knockout because the endogenous gene is replaced, but in the case of a transplacement the replacement is by another version of the same gene. After test substances have been administered to the animals, the the diabetic phenotypemust be assessed. If the test substance prevents or suppresses thethe diabetic phenotype, then the test substance is a candidate therapeutic agent for the treatment of diabetes.. These animal models provide an extremely important testing vehicle for potential therapeutic products.

In one embodiment of the invention, transgenic animals are produced which contain a functional transgene encoding a functional AGT polypeptide or variants thereof. Transgenic animals expressing *AGT* transgenes, recombinant cell lines derived from such animals and transgenic embryos may be useful in methods for screening for and identifying agents that induce or repress function of AGT. Transgenic animals of the present invention also can be used as models for studying indications such as IDDM.

In one embodiment of the invention, a *AGT* transgene is introduced into a non-human host to produce a transgenic animal expressing a human, murine or other species *AGT* gene. The transgenic animal is produced by the integration of the transgene into the genome in a

manner that permits the expression of the transgene. Methods for producing transgenic animals are generally described by Wagner and Hoppe (U.S. Patent No. 4,873,191; which is incorporated herein by reference), Brinster *et al.* 1985; which is incorporated herein by reference in its entirety) and in "Manipulating the Mouse Embryo; A Laboratory Manual" 2nd edition (eds., Hogan, Beddington, Costantimi and Long, Cold Spring Harbor Laboratory Press, 1994; which is incorporated herein by reference in its entirety).

It may be desirable to replace the endogenous *AGT* by homologous recombination between the transgene or a mutant gene and the endogenous gene; or the endogenous gene may be eliminated by deletion as in the preparation of "knock-out" animals. Typically, a *AGT* gene flanked by genomic sequences is transferred by microinjection into a fertilized egg. The microinjected eggs are implanted into a host female, and the progeny are screened for the expression of the transgene. Transgenic animals may be produced from the fertilized eggs from a number of animals including, but not limited to reptiles, amphibians, birds, mammals, and fish. Within a particularly preferred embodiment, transgenic mice are generated which overexpress AGT or express a mutant form of the polypeptide. Alternatively, the absence of a *AGT* in "knock-out" mice permits the study of the effects that loss of AGT protein has on a cell *in vivo*. Knock-out mice also provide a model for the development of AGT-related diabetes.

Methods for producing knockout animals are generally described by Shastry (1995, 1998) and Osterrieder and Wolf (1998). The production of conditional knockout animals, in which the gene is active until knocked out at the desired time is generally described by Feil et al. (1996), Gagneten et al. (1997) and Lobe and Nagy (1998). Each of these references is incorporated herein by reference.

As noted above, transgenic animals and cell lines derived from such animals may find use in certain testing experiments. In this regard, transgenic animals and cell lines capable of expressing wild-type or mutant AGT may be exposed to test substances. These test substances can be screened for the ability to alter expression of wild-type *AGT* or alter the expression or function of mutant AGT.

Pharmaceutical Compositions and Routes of Administration

The AGT polypeptides, antibodies, peptides and nucleic acids of the present invention can be formulated in pharmaceutical compositions, which are prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA). The composition may contain the active agent or pharmaceutically acceptable salts of the active agent. These compositions may

comprise, in addition to one of the active substances, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral, intrathecal, epineural or parenteral.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, WO 96/11698.

For parenteral administration, the compound may be dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

The active agent is preferably administered in a therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc., is within the responsibility of general practitioners or specialists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in *Remington's Pharmaceutical Sciences*.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell

specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

Instead of administering these agents directly, they could be produced in the target cell, e.g. in a viral vector such as described above or in a cell based delivery system such as described in U.S. Patent No. 5,550,050 and published PCT application Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635, designed for implantation in a patient. The vector could be targeted to the specific cells to be treated, or it could contain regulatory elements which are more tissue specific to the target cells. The cell based delivery system is designed to be implanted in a patient's body at the desired target site and contains a coding sequence for the active agent. Alternatively, the agent could be administered in a precursor form for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. See for example, EP 425,731A and WO 90/07936.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are within the skill of the art. See, e.g., Maniatis *et al.*, 1982; Sambrook *et al.*, 1989; Ausubel *et al.*, 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991; Harlow and Lane, 1988; Jakoby and Pastan, 1979; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu et al. eds.), *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Hogan et al., Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).. A general discussion of techniques and materials for human gene mapping, including mapping of human chromosome 1, is provided, e.g., in White and Lalouel, 1988.

EXAMPLES

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized.

EXAMPLE 1

Methods

Subjects, genotyping, and haplotype analyses. The families used in this study are part of a collection of IDDM families at the Human Biological Data Interchange (HBDI), Philadelphia, PA. Genomic DNA samples were ordered from the HBDI Repository located at the Coriell Institute for Medical Research in Camden, NJ. In the initial part of this study samples were ordered for individuals from 38 nuclear families with 3 siblings affected with IDDM. After identification of sequence variants in this first set of families, an additional 308 unrelated individuals with IDDM were obtained from HBDI. The initial control set of 120 samples were the grandparents of the Utah families included in the Centre d'Etude du Polymorphisme Humain (CEPH) database. Unrelated spouses from other Utah families were used as additional controls. No information on the presence or absence of diabetes in these samples was available. Genotypes using fluorescently labelled primers were determined for 11 simple tandem repeat polymorphisms spanning the published IDDM linkage on chromosome 1q (Concannon et al., 1998). Marker positions and primer sequences were obtained from Genethon (*D1S237, D1S469, D1S439, D1S479, D1S437, D1S225, D1S251, D1S1568, AFMa072xa5x1*), Marshfield (*D1S320*), and CHLC (*D1S1621*). Haplotype analysis was performed by using the program MCLINK (Thomas et al., 1999). Comparisons of observed frequencies of mutation in cases and controls were made with a chi-squared, log likelihood ratio, test. The null hypothesis is that the observations come from Binomial distributions with the same probability parameters. The p-value quoted for each comparison is that for rejection of the null hypothesis in favor of the alternative hypothesis that the Binomials have unequal probability parameters.

Genomic sequencing and mutation detection. A human genomic BAC library (Research Genetics) was screened by PCR for clones containing the marker *WI18298*. The end sequences of BAC 1-30 were used as probes to assemble a BAC contig. 6x genomic sequencing coverage of BACs *1-30, 1-104* and *1-112* was obtained by sequencing of plasmid libraries constructed with randomly sheared and *Sau*3A-digested BAC DNA. For mutation detection exons were

amplified from genomic DNA with primers placed in intronic sequences flanking each exon. Nested amplifications were performed with primers containing M13 forward (5'-GTTTTCCCAGTCACGACG-3'; SEQ ID NO:9) and reverse (5'-AGGAAACAGCTATGAC CAT-3'; SEQ ID NO:10) universal primer sequences at the 5' termini. Nested PCR products were cycle sequenced directly and run on ABI 377 automated sequencers (Perkin-Elmer). Sequence data were analyzed for polymorphisms using software developed at Myriad Genetics, Inc.

GenBank accession numbers. Human *AGT* retina EST, AA059356; human *AGT* M24689; rat *AGT* L00094; sheep *AGT* D17520; mouse *AGT* AF045887; gorilla *AGT* 188488; chimpanzee *AGT* 188487. The sequences of AGT for gorilla and chimpanzee were generated in this study.

EXAMPLE 2

Association of AGT Gene and IDDM

To investigate whether mutations in *AGT* are responsible for the reported linkage of type 1 diabetes to the chromosomal region 1q42-43, we carried out a three stage analysis. Initially we obtained a set of 38 nuclear families, each with three children affected with type 1 diabetes from the Human Biological Data Interchange. These were screened to identify potentially deleterious mutations. We then screened independent sets of 308 unrelated cases and 1003 controls to determine whether the identified mutations were more common in cases than controls. Finally we screened all 308 cases and 631 of the 1003 controls to determine whether rare polymorphisms, potential causal mutations, were more common in cases than controls. In order to select from the 38 initial families those that could potentially segregate a mutation among all affected members, each family was genotyped using 11 microsatellite markers spanning a 32 cM region surrounding the *AGT* locus on chromosome 1 (Fig. 1). This revealed that in 20 of the 38 families the affected individuals shared a haplotype in part of the 32 cM region.

The entire coding sequence, intron-exon boundaries, and 5' and 3' untranslated regions of *AGT* were screened for sequence variants in the genomic DNA of one affected member from each of the 20 nuclear families. Genomic DNA was amplified using primers positioned in introns flanking each exon of *AGT* (Table 1). Three families each contained a unique polymorphism in *AGT*, which we later determined segregated into all affected individuals in the family.

TABLE 1

Primers Used in PCR Amplification for Mutation Detection

Exon	PCR*	Forward primer (SEQ ID NO:)	Reverse primer (SEQ ID NO:)
alternative exon I	primary	5'-CCTCGAAGTGGCCTATGGAA-3' (11)	5'-GCGAGACTCTATGGGTGATG-3' (12)
	secondary	5'-GACTGAGACTCTCCTGTTTC-3' (13)	5'-CGGGGTGGCATCTTCAGTC-3' (14)
	secondary	5'-TGGCACATTCTGCTCCTTAG-3' (15)	5'-ATACAGCGAGTCCTGGGTCT-3' (16)
	secondary	5'-GACCAGGCAGTTGAGGAAGT-3' (17)	5'-GATTCACACTCTCACGCTGC-3' (18)
promoter/5' genomic	primary	5'-CCACTTTCCAGCATGTTACC-3' (19)	5'-CTGCTATTCCTCCTGATGGC-3' (20)
	secondary	5'-ACAGGCCCATGTGAGATATG-3' (21)	5'-TGGTATTAGGGCACTGAAGG-3' (22)
	secondary	5'-TGTGTTGAAGCAGGATCTTC-3' (23)	5'-GATGCAGGCATTGAAAGATG-3' (24)
	secondary	5'-GTGTTTAACAGTCTCCCCAGC-3' (25)	5'-CTAGGTGTGTGACAGCCTGA-3' (26)
	secondary	5'-CATGTCCCTGTGGCCTCTT-3' (27)	5'-TGAGGGGTGGGGATGGAG-3' (28)
	secondary	5'-CTGGTCATGTGAAACTTACC-3' (29)	5'-ACCTTCAGCTGCTCCAAAGA-3' (30)
exon I	primary	5'-CCACTTTCCAGCATGTTACC-3' (31)	5'-CTGCTATTCCTCCTGATGGC-3' (32)
	secondary	5'-AACCCTCCTCTCCAGCCT-3' (33)	5'-TCATCACCGTGCCTCCTC-3' (34)
exon II	primary	5'-TACTTGGACTTTGGGCTGAG-3' (35)	5'-TTGAGCAAGGCACTTTGTTTC-3' (36)
	secondary	5'-GGCTAAATGGTGACAGGGAAT-3' (37)	5'-TAGGGCCTTTTCATCCACAG-3' (38)
	secondary	5'-AATGCCGGGAAGCCCAAAGA-3' (39)	5'-TCCAAGGCTCCCAGATAGAG-3' (40)
	secondary	5'-CTCTCCCCAACGGCTGTCTT-3' (41)	5'-CTGCACAAACGGCTGCTTCA-3' (42)
	secondary	5'-CTCTATCTGGGAGCCTTGGA-3' (43)	5'-CACAGCCTGCATGAACCTGT-3' (44)
	secondary	5'-TGAAGCAGCCGTTTGTGCAG-3' (45)	5'-TCCCCACTTCTCAAGGGTG-3' (46)
	secondary	5'-ACAGGTTCATGCAGGCTGTG-3' (47)	5'-TCCCCACTTCTCAAGGGTG-3' (48)
exon III	primary	5'-TGCCTCAGTGAACTCAAATGG-3' (49)	5'-CTCCCCCTCCAAAACTACCA-3' (50)
	secondary	5'-CAGGAGAATGCAGAGTGGC-3' (51)	5'-GCAGGCGCTCTCAGTGAAG-3' (52)
	secondary	5'-AGCACTGGAGTGACATCCAG-3' (53)	5'-GATGGAGGACTGGTAGACAG-3' (54)
exon IV	primary	5'-AAGGGGGAGGATGACTTAGT-3' (55)	5'-GAAATTCCATCCAAAGTCCAG-3' (56)
	secondary	5'-GTGCAAGGAGCATAAGCCTG-3' (57)	5'-CTACTCTCCGCTCCCTCTT-3' (58)
exon V	primary	5'-AAGGGGGAGGATGACTTAGT-3' (59)	5'-GAAATTCCATCCAAAGTCCAG-3' (60)
	secondary	5'-TAGCCCTCAGCACCCTG-3' (61)	5'-TGTCCGGGGTTGTTATCTG-3' (62)
	secondary	5'-AGAACACAGTGCCTGGCAAG-3' (63)	5'-TTCACAAACAAGCTGGTCGG-3' (64)

	secondary	5'-AGTGTTTAGCGCGGGACTAC-3' (65)	5'-TTTGGAGGCTTATTGTGGCA-3' (66)
	secondary	5'-TGAAAGATGCAAGCACCTGA-3' (67)	5'-GAAAGCACTTTCGTTTGCAC-3' (68)

* Secondary primers had M13 forward and reverse universal sequences added to the 5' end.

In family 24, a T to C transition was found in exon 2 at nucleotide 190 in the cDNA, which resulted in a substitution of arginine for cysteine at position 18 in the angiotensinogen mature peptide (Fig. 2a; Fig. 3a; 3b). Cys18 is one of two conserved cysteine residues in mammalian angiotensinogen(s) (Fig. 3) that has been shown *in vitro* to form a disulfide bond with Cys138 (Gimenez-Roqueplo et al., 1998a; Streatfeild-James et al., 1998). Disruption of disulfide bond formation might affect an unknown role for *AGT* in diabetes. Although it seems likely that disulfide bond formation contributes to a structurally constrained N-terminus (Streatfeild-James et al., 1998), disruption of the disulfide bond by *in vitro* mutagenesis of Cys18 did not affect the rate of angiotensinogen cleavage by renin (Gimenez-Roqueplo et al., 1998a). However, under physiological conditions, alteration of the protein's structure might disrupt association with other binding partners that would then indirectly affect cleavage by renin (Inoue et al., 1997). It is also possible that mutation of Cys18 indirectly affects glycosylation of angiotensinogen. The partner with which Cys18 forms a disulfide bond, Cys138, lies in the middle of the N-glycosylation site Asn137-Cys138-Thr139 (Fig. 3b). Of the four N-glycosylation sites in angiotensinogen, Asn137 is the least glycosylated (Gimenez-Roqueplo et al., 1998b). In the absence of disulfide bond formation at Cys138, Asn137 might be glycosylated more frequently. Mutations in *AGT* that lead to increased glycosylation affect the amount of angiotensinogen secreted, and therefore indirectly the amount of Ang I (the peptide cleaved from *AGT* by renin and further converted to Ang II) produced (Gimenez-Roqueplo et al., 1996). Consequently, Cys18Arg would cause a decrease in Ang I formation.

Mutations detected in family 19 and family 30 were both in a 5' untranslated region of *AGT*. An alternative 5' exon of *AGT* has been reported in the EST database. This alternate form was verified by PCR and sequencing in this study. We determined from our BAC contig that the alternative exon lies at least 100kb upstream of the published exon I (Fig. 1, Fig. 3), and it was only detected by PCR in retina cDNA. The alternative exon that splices into exon II does not contain an open reading frame that extends out of the exon. Translation of the alternative transcript is predicted to begin at the first methionine in exon II (Fig. 3a) as previously reported (Gaillard et al., 1989). Although the alternative transcript produces the same angiotensinogen polypeptide as the published *AGT* cDNA, its expression could be regulated in a tissue-specific manner. It is not known what effect variants in the 5' untranslated region would have on the expression or stability of the transcript. A C to T transition at nucleotide 96 in the exon was seen in all affected individuals in family 19 (Fig. 2b; Fig. 3a). In family 30, a T to C transition at nucleotide 286 in this exon segregated with the disease (Fig. 2c; Fig. 3a).

Family 30 also contained a C to T transition at nucleotide 928 in exon 3, resulting in a non-conservative substitution of serine for proline at position 264 (Fig. 3a, 3c). This position is a proline in hominoids and a hydrophobic amino acid in other non-primate species (Fig. 3c); therefore the substitution of a hydrophilic amino acid (serine) is expected to have profound structural consequences. The Pro264Ser missense mutation is linked to a silent G to A transition at the preceding nucleotide, as well as to a C to T transition at –811 in the upstream genomic sequence (shown in SEQ ID NO:1). This haplotype of three linked polymorphisms (C-811T, G927A and Pro264Ser) did not segregate into all the affected individuals in family 30 (Fig. 2d, 2e). However, this is not unexpected given that type 1 diabetes is a complex trait, and that predisposition to the disease probably involves a combination of mutations in multiple genes. If carrying a subset of these mutated genes results in the diabetic phenotype, members of the same family that do not share at the *AGT* locus could have an overlapping set of mutations in other gene(s).

EXAMPLE 3

Identification of Additional Polymorphisms

If the *AGT* polymorphisms identified in Example B are truly predisposing mutations, they should be detected in other type 1 diabetic individuals and should be rare or absent in controls. To identify additional mutations, a set of DNA samples from 308 unrelated type 1 diabetic individuals was screened for sequence variants in *AGT*. These cases were selected from 308 additional families in the Human Biological Data Interchange. A set of control samples was screened in the same way for statistical comparison (see Example 1 and Table 2). The Cys18Arg mutation was observed three times in the new case set. It segregated with the disease in family 164, but not in families 214 and 344 (Table 2). The Cys18Arg mutation was observed in only 1 of 1003 control samples, indicating that it is enriched in the disease set (3 of 308 vs. 1 of 1003, p =0.03).

TABLE 2

AGT Polymorphisms in Type 1 Diabetics

Family ID	HBDI ID	Exon	Nucleotide change[a]	Change in *AGT* polypeptide	Segregation among affecteds	Observations in cases	Observations in controls[b]
54	B550	alternative I[1]	G(26)T	5' UTR	1/2	1/328	0/639
19	DCCT 20008	alternative I	C(96)T	5' UTR	3/3	1/328	0/639
30	CD2 2	alternative I	T(286)C	5' UTR	3/3	1/328	0/639
30	CD2 2	5' genomic[c,2]	C(-811)T[d]	-	2/3	3/328	1/635
243	A311	"	"	-	1/2	"	"
329	CD328	"	"	-	2/2	"	"
114	ENG8	5' genomic[c]	T(-741)C[e]	-	2/2	1/328	0/635
114	ENG8	5' genomic[c]	ΔG(-603)[e]		2/2	1/328	0/635
114	ENG8	5' genomic[c]	C(-281)T[e]	-	2/2	1/328	0/631
266	DCCT 17123	5' genomic[c]	A(-715)G		1/2	1/328	0/635
162	NM41	5' genomic[c]	T(-363)A	-	1/2	1/328	0/633
339	PM06	5' genomic[c]	G(-259)A		2/2	1/328	0/631
236	A342	5' genomic[c]	T(-253)C	-	2/2	1/328	0/631
150	NM23A	5' genomic[c]	G(-183)A	-	2/2	1/328	0/631
24	NM61	II	T(190)C[3]	Cys18Arg[4]	4/4	4/328	1/1003
164	NM49	"	"	"	2/2	"	"
214	C241	"	"	"	1/2	"	"
344	DE3SAM3	"	"	"	1/2	"	"
305	E428	II	C(503)T	Ala122Val	2/2	1/328	0/638
348	NM42	II	A(881)G	Tyr248Cys[f]	1/2	1/328	2/638
30	CD2 2	III	G(927)A[d]	silent	2/3	2/328	0/1003
243	A311	"	"	"	1/2	"	"
30	CD2 2	III	C(928)T[d]	Pro264Ser	2/3	2/328	0/1003
243	A311	"	"	"	1/2	"	"

318	PP 1	III	G(1093)A	Asp319Asn	1/2	1/328	0/628
119	REQ380	IV	C(1213)A[g]	Leu359Met	1/2	1/328	0/635
272	DCCT 19301	V	C(1340)T	Ala401Val	1/2	1/328	0/636
229	NM68	V	G(1529)T	3' UTR	3/3	1/328	1/636

[a] The number in parenthesis refers to the position of the nucleotide change relative to the start site of transcription, or the position in the alternative I exon as described in further detail herein.

[b] Total 639 controls were tested for each polymorphism, the number is <639 due to random sample failure. For the two recurrent mutations, additional control samples were tested.

[c] Refers to the genomic sequence upstream of the published exon 1.

[d] The C(-811)T, G(927)A and C(928)T polymorphisms are linked in families 30 and 243.

[e] The T(-741), ΔG(-603) and C(-281)T polymorphisms are linked in family 114.

[f] This polymorphism was previously shown to affect the rate of angiotensinogen production in hypertensive individuals (Gimenez-Roqueplo et al., 1996).

[g] This polymorphism was reported previously in a hypertensive individual (Jeunemaitre, 1992).

[1] Position in alternative I refers to position within alternative exon 1 of SEQ ID NO:8, i.e. nucleotide 26 of alternative exon 1 is at position 176 of SEQ ID NO:8.

[2] Position in 5' genomic refers to position 5' of exon 1 of SEQ ID NO:1 where exon 1 starts at position 3012, i.e. nucleotide -811 of 5' genomic is at position 2201 of SEQ ID NO:1.

[3] Position with respect to exons II-V refer to nucleotide position in SEQ ID NO:6.

[4] Position with respect to amino acids refers to position in mature AGT peptide, i.e., the peptide of SEQ ID NO:8 having the signal peptide (residues 1-33) removed.

In the new case set the Pro264Ser mutation was seen once, with its accompanying polymorphisms, but was not seen in the controls (1/308 vs. 0/1003, p =0.09). The C-811T polymorphism was observed once in the controls but in isolation, not as part of the C-811T, G927A and Pro264Ser haplotype. The G297A change was not seen in the controls.

A number of novel polymorphisms in *AGT* were detected in the second set of unrelated diabetic individuals. Segregation analyses showed that about 50% of the polymorphisms segregated into all affected individuals within a family (Table 2). An individual in family 54 contained a G to T transversion at nucleotide 26 in alternative exon I. Within the genomic sequence upstream of exon I, the sequence variants A(-715)G, T(-363)A, G(-259)A, T(-253)C and G(-183)A were detected in individuals from families 266, 162, 339, 236 and 150, respectively. Three other polymorphisms in the upstream genomic sequence, T(-741)C, ΔG(-603) and C(-281)T, were linked in family 114. Although each of these changes lies upstream of the characterized proximal promoter in *AGT* (Brasier et al., 1999), they could potentially alter *AGT* expression by affecting promoter elements not yet identified. Recently, Yanai et al. (1999) have reported a role for hepatocyte nuclear factor 4 (HNF4) and chicken ovalabumin upstream pormoter transcription factor (COUP-TFII) in regulating transcription of the angiotensinogen gene. Truncating mutations in the HNF4 gene were found in patients with maturity onset diabetes of the youn, and the HNF4 gene is therefor also called MODY1 (Yamagata et al., 1996; Lidner et al., 1997). One of the DNA fragments identified as an HNF4 activating element by deletion analysis is the fragment from –242 to –295. Three of the variants in the sequence upstream of exon 1 are within this fragment (T(-253)C in family 236, G(-259)A in family 339 and C(-281)T in family 114). None of these variants was detected in 631 controls.

Three previously unreported missense mutations were detected in the coding sequence of *AGT*. An Ala122Val missense mutation was detected in family 305, an Asp319Asn was present in family 318, and an Ala401Val was found in family 272. Of these, only Ala122Val segregated with the disease in the family. Considering the rare missense mutations which were detected in *AGT*, five are at positions highly conserved in primates (Cys18Arg, Ala122Val, Pro264Ser, Asp319Asn, and Ala401Val, see Fig. 3b, 3c). In the 3' UTR a G to T transversion 35 bp downstream of the translation stop (G1530T) was observed in an individual from family 229. The discovery of enhancer elements in exon 5 and in the genomic sequence downstream of *AGT* (Nibu et al., 1994a; Nibu et al., 1994b) suggests this change might affect *AGT* expression. This change was detected once in 636 controls. Finally, two missense mutations that had been identified previously in hypertensive individuals were seen once in the set of type 1 diabetics. A

Tyr248Cys mutation was detected in an individual from family 348. This change was previously reported to affect the rate of angiotensinogen production in hypertensive individuals (Gimenez-Roqueplo et al., 1996). An individual in family 119 contained a Leu359Met substitution that had been shown previously in a hypertensive patient (Jeunemaitre et al., 1992). Both changes were also found in our control samples (Table 2). Neither of these missense changes segregated with diabetes in these families.

All of the coding and nod-coding regions of *AGT* which were screened for polymorphisms in type 1 diabetics were also sequenced in 631 control individuals. Several polymorphisms were seen in the controls that were not seen in the cases (see Table 3). The frequency of rare changes, defined as present at less than 5 times in all samples, was compared in cases and controls. Fewer control samples carried rare changes when compared to type I diabetic cases (20 of 308 vs. 17 of 631, p=0.007). It is interesting to note that there were no polymorphisms detected in the sequence upstream of exon 1 between –64 and –413 in the 631 controls. There were five polymorphisms detected in this region in the type 1 diabetic cases including the three changes found in the fragment involved in activation of transcription of *AGT* by HNF4.

TABLE 3

AGT Rare Changes in Controls

Control Sample #	Exon	Nucleotide change[a]	Corresponding change in AGT peptide	Amino Acid Conserved?	Observations in controls[b]	Observations in Type I cases
OS-93	Alternative exon I	C(257)T[1]	5' untranslated region	-	1/639	0/328
CA-158	Alternative exon I	G(283)A	5' untranslated region	-	1/639	0/328
CA-63	Upstream genomic[c]	A(-854)T[2]	-	-	1/635	0/328
CA-103	Upstream genomic[c]	C(-811)T	-	-	1/635	3/328
CEPH-57	Upstream genomic[c]	C(-563)T	-	-	1/633	0/328
CEPH-67	Upstream genomic[c]	C(-415)T	-	-	1/633	0/328
CA-153	Upstream genomic[c]	C(-413)A	-	-	1/633	0/328
CA-172	Upstream genomic[c]	G(-63)C	-	-	1/631	0/328
MY-37	Exon II	T(190)C[3]	Cys(18)Arg[4]	Yes	1/624	4/328
CA-84	Exon II	G(286)A	Ala(50)Thr	No, Thr in *Rattus&Mus*	1/635	0/328
CA-74	Exon II	G(379)T	Gly(81)Cys	Yes	1/635	0/328
CA-199	Exon II	C(748)T	Arg(204)Cys	Yes	1/638	0/328
AS-138	Exon II	A(881)G	Tyr(248)Cys[d]	Yes	2/638	1/328
CA-197	Exon II	A(881)G	Tyr(248)Cys[d]	Yes	2/638	1/328
CA-68	Exon IV	C(1213)A	Leu(359)Met[e]	Yes	1/635	1/328

CA-139	Exon IV	G(1246)A	Ala(370)Thr	No, Thr in all other species	1/639	0/328
AS-51	Exon V	G(1469)A	Arg(444)His	No, Valine in ovis	1/636	0/328

[a] The number in parenthesis refers to the position of the nucleotide change relative to the start site of transcription, or the position in the alternative I exon as further described herein.

[b] Total 639 controls were tested for each polymorphism, the number is <639 due to random sample failure.

[c] Refers to the genomic sequence upstream of the published exon 1.

[d] This polymorphism was previously shown to affect the rate of angiotensinogen production in hypertensive individuals (Gimenez-Roqueplo et al., 1996).

[e] This polymorphism was reported previously in a hypertensive individual (Jeunemaitre et al., 1992).

[1] Position in alternative I refers to position within alternative exon 1 of SEQ ID NO:8, i.e. nucleotide 26 of alternative exon 1 is at position 176 of SEQ ID NO:8.

[2] Position in 5' genomic refers to position 5' of exon 1 of SEQ ID NO:1 where exon 1 starts at position 3012, i.e. nucleotide -811 of 5' genomic is at position 2201 of SEQ ID NO:1.

[3] Position with respect to exons II-V refer to nucleotide position in SEQ ID NO:6.

[4] Position with respect to amino acids refers to position in mature AGT peptide, i.e., the peptide of SEQ ID NO:8 having the signal peptide (residues 1-33) removed.

To conclude, the nature of the polymorphisms found in an initial set of diabetic families, their combined frequency in an independent set of cases compared with that in a set of controls (4/308 vs. 1/1003, p=0.008), and the abundance of rare mutations in type I diabetic cases all indicate that mutations in *AGT* increases susceptibility to the disease. The identification of missense mutations confirms the importance of the mature part of the protein in *AGT* structure and function. Novel polymorphisms in the alternative 5' exon could provide clues to tissue-specific functions of the renin-angiotensin system. The role of *AGT* in the microvascular complications of diabetes (Krolewski et al., 1998; Rogus et al., 1998) can be further considered. Perhaps the most interesting prospect is that our results in conjunction with the recent demonstration of angiotensin II receptors on cells of the islets of Langerhans that also stain for insulin, implies a direct role for *AGT* in pancreatic β-cell function (Tahmasebi et al., 1999). Understanding the contribution of *AGT* to type 1 diabetes could lead to new treatments and preventative medicines for the disease.

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

LIST OF REFERENCES

Anand, R. (1992). *Techniques for the Analysis of Complex Genomes*, (Academic Press).

Ausubel, F.M., *et al.* (1992). *Current Protocols in Molecular Biology*, (J. Wiley and Sons, NY).

Bartel, P.L., *et al.* (1993). "Using the 2-hybrid system to detect protein-protein interactions." *In:* Cellular Interactions in Development: A Practical Approach, Oxford University Press, pp. 153-179.

Bell, G. et al. (1984). *Diabetes* **33**:176.

Borman S (1996). *Chemical & Engineering News*, December 9 issue, pp. 42-43.

Botstein, *et al.* (1980). *Am. J. Hum. Genet.* **32**:314-331.

Capecchi, M.R. (1989). *Science* **244**:1288.

Cariello (1988). *Human Genetics* **42**:726.

Chee M, et al. (1996). *Science* 274:610-614.

Chevray, P.M. and Nathans, D.N. (1992). *Proc. Natl. Acad. Sci. USA* **89**:5789-5793.

Conner, B.J., *et al.* (1983). *Proc. Natl. Acad. Sci. USA* **80**:278-282.

Cornall, R.J. et al. (1991) *Nature* **353**:262.

Cotton, *et al.* (1988). *Proc. Natl. Acad. Sci. USA* **85**:4397-4401.

Davies, J.L. et al. (1994). *Nature* **371**:130-136.

De Gouyon, B. et al. (1993). *Proc. Nat. Acad. Sci. USA* **90**:1877.

DeRisi J, et al. (1996). *Nat. Genet.* 14:457-460.

Donehower, L.A., *et al.* (1992). *Nature* **356**:215.

Erickson, J. *et al.*, (1990). *Science* **249**:527-533.

Feil et al., (1996). *Proc. Natl. Acad. Sci. USA* **93**:10887-10890.

Fields, S. and Song, O-K. (1989). *Nature* **340**:245-246.

Finkelstein, J., *et al.* (1990). *Genomics* **7**:167-172.

Fodor, S.P.A. (1997). DNA Sequencing. Massively Parallel Genomics. *Science* **277**:393-395.

Froguel, P. et al. (1992). *Nature* **356**:162-164.

Froguel, P. et al. (1993). *N. Engl. J. Med.* **328**:697-702.

Gagneten et al. (1997). *Nucl. Acids Res.* **25**:3326-3331.

Garchon, H.J. et al. (1991). *Nature* **353**:260.

Glover, D. (1985). *DNA Cloning*, **I** and **II** (Oxford Press).

Grompe, M., (1993). *Nature Genetics* **5**:111-117.

Grompe, M., *et al.*, (1989). *Proc. Natl. Acad. Sci. USA* **86**:5855-5892.

Guthrie, G. and Fink, G.R. (1991). *Guide to Yeast Genetics and Molecular Biology* (Academic Press).

Hacia JG, et al. (1996). *Nature Genetics* 14:441-447.

Harlow and Lane (1988). Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Harris, C.L. et al. (1996). *Nature Genet.* **13**:161-166.

Harris, M.I. et al. (1987). *Diabetes* **36**:523-534.

Harris, M.I., et al. (1992) *Diabetes Care* **15**:815-819

Hasty, P., K., *et al.* (1991). *Nature* **350**:243.

Hattersley, A.T. et al. (1992). *Lancet* **339**:1307-1310.

Hodgson, J. (1991). *Bio/Technology* **9**:19-21.

Hogan et al. (eds) (1994). *Manipulating the Mouse Embryo: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Jablonski, E., *et al.* (1986). *Nuc. Acids Res.* **14**:6115-6128.

Jakoby, W.B. and Pastan, I.H. (eds.) (1979). *Cell Culture. Methods in Enzymology*, Vol. 58 (Academic Press, Inc., Harcourt Brace Jovanovich (NY).

Jeffreys, *et al.* (1985). *Nature* **314**:67-73.

Kadowaki et al. (1994). *N. Engl. J. Med.* **330**:962-968.

King, H. and Zimmer, P. (1988). *Wld Hlth. Statist. Quart.* **41**:190-196;

Kinszler, K.W., *et al.* (1991). *Science* **251**:1366-1370.

Kinzler, K.W. and Vogelstein, B. 1997. *Nature* **386**: 761-763.

Kozlowski et al. (1993). *J. Recept. Res.* **13**:1031-40.

Landegren, *et al.* (1988). *Science* **242**:229.

Lee, J.E., *et al.* (1995). *Science* **268**:836-844.

Lindner, T. et al. (1997). *J. Clin. Invest.* **100**:1400-1405.

Lipshutz RJ, et al. (1995). *BioTechniques* 19:442-447.

Litt, *et al.* (1989). *Am. J. Hum. Genet.* **44**:397-401.

Lobe and Nagy (1998). *Bioessays* **20**:200-208.

Lockhart DJ, et al. (1996). *Nature Biotechnology* 14:1675-1680.

Mahtani, M.M. et al. (1996). *Nature Genetics* **14**:90-94.

Maniatis. T., *et al.* (1982). *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Matthews and Kricka (1988). *Anal. Biochem.* **169**:1.

Mittlin (1989). *Clinical Chem.* **35**:1819.

Modrich, P. (1991). *Ann. Rev. Genet.* **25**:229-253.

Mombaerts, P., *et al.* (1992). *Cell* **68**:869.

Morahan, G. et al. (1994). *Proc. Nat. Acad. Sci. USA* **91**:5998

Nakamura, *et al.* (1987). *Science* **235**:1616-1622.

Newton, C.R., et al. (1989). *Nucl. Acids Res.* **17**:2503-2516.

Nguyen, Q., *et al.* (1992). *BioTechniques* **13**:116-123.

Novack, *et al.* (1986). *Proc. Natl. Acad. Sci. USA* **83**:586.

Orita, *et al.* (1989). *Proc. Natl. Acad. Sci. USA* **86**:2776-2770.

Osterrieder and Wolf (1998). *Rev. Sci. Tech.* **17**:351-364.

Philpott, K.L., *et al.* (1992). *Science* **256**:1448.

Prochazka, M. et al. (1987). *Science* **237**:286.

Rano and Kidd (1989). *Nucl. Acids Res.* **17**:8392.

Rigby, P.W.J., *et al.* (1977). *J. Mol. Biol.* **113**:237-251.

Rosa et al (1998). *FEBS Letters* **436**:267-270.

Rotter et al. (1990). *Diabetes Mellitus: Theory and Practice*, 378-413.

Samanen et al. (1991). *J. Med. Chem.* **34**:3036-43.

Sambrook, J., *et al.* (1989). *Molecular Cloning: A Laboratory Manual*, 2nd Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Serreze, D. et al. (1994). *J. Exp. Med.* **180**:1553.

Shastry et al. (1995). *Experientia* **51**:1028-1039.

Shastry et al. (1998). *Mol. Cell. Biochem.* **181**:163-179.

Sheffield, V.C., *et al.* (1989). *Proc. Natl. Acad. Sci. USA* **86**:232-236.

Sheffield, V.C., *et al.* (1991). *Am. J. Hum. Genet.* **49**:699-706.

Shenk, *et al.* (1975). *Proc. Natl. Acad. Sci. USA* **72**:989.

Shinkai, Y., *et al.* (1992). *Cell* **68**:855.

Shoemaker DD, et al. (1996). *Nature Genetics* 14:450-456.

Skolnick, M.H. and Wallace, B.R. (1988). *Genomics* **2**:273-279.

Snouwaert, J.N., *et al.* (1992). *Science* **257**:1083.

Steiner et al. (1990). *Diabetes Care* **13**:600-609.

Tavtigian, S., *et al.* (1996). *Nature Genetics* **12**:333-337.

Taylor et al. (1992). *Endocrine Rev.* **13**:566-595.

Todd, J.A. (1990). *Immunol. Today* **11**:122.

Todd, J.A., et al. (1991). *Nature* **351**:542.

Van den Ouwenland, J.M.W. et al. (1992). *Nature Genet.* **1**:368-371.

Verge, C.F. et al. (1998). *J. Clin. Invest.* **102**:1569-1575.

Wartell, R.M., *et al.* (1990). *Nucl. Acids Res.* **18**:2699-2705.

Weber and May (1989). *Am. J. Hum. Genet.* **44**:388-396.

Wells, J.A. (1991). *Methods in Enzymol.* **202**:390-411.

White, M.B., *et al.*, (1992). *Genomics* **12**:301-306.

White and Lalouel (1988). *Ann. Rev. Genet.* **22**:259-279.

Yamagata, K. et al. (1996). *Nature* **384**:458-460.

Yanai, K. et al. (1999). *J. Biol. Chem.* **274**:34605-34612.

List of Patents and Patent Applications:

U.S. Patent No. 4,376,110.

U.S. Patent No. 4,486,530.

U.S. Patent No. 4,868,105.

U.S. Patent No. 4,873,191.

U.S. Patent No. 5,093,246.

U.S. Patent No. 5,550,050.

U.S. Patent No. 5,800,998.

U.S. Patent No. 5,837,492.

U.S. Patent No. 5,891,628.

EPO Publication No. 225,807

EP 425,731A.

European Patent Application Publication No. 0332435

Geysen, H., PCT published application WO 84/03564, published 13 September 1984

WO 90/07936.

WO 92/19195.

WO 93/07282

WO 94/25503.

WO 95/01203.

WO 95/05452.

WO 96/02286.

WO 96/02646.

WO 96/11698.

WO 96/40871.

WO 96/40959.

WO 97/12635.

WHAT IS CLAIMED IS:

1. A nucleic acid selected from the group consisting of:

(a) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, or complement thereof;

(b) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:2, or complement thereof;

(c) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:3, or complement thereof;

(d) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:4, or complement thereof;

(e) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:5, or complement thereof; and

(f) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:8, or complement thereof.

2. A nucleic acid selected from the group consisting of:

(a) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:8, wherein G at nucleotide position 26 of the exon is substituted with T, or a complement thereof;

(b) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:8, wherein C at nucleotide position 96 of the exon is substituted with T, or a complement thereof;

(c) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:8, wherein T at nucleotide position 286 of the exon is substituted with C, or a complement thereof;

(d) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein C at nucleotide position 2201 is substituted with T, or a complement thereof

(e) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein T at nucleotide position 2271 is substituted with C, or a complement thereof

(f) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein G at nucleotide position 603 is deleted, or a complement thereof

(g) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein C at nucleotide position 2731 is substituted with T, or a complement thereof

(h) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein A at nucleotide position 2297 is substituted with G, or a complement thereof

(i) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein T at nucleotide position 2649 is substituted with A, or a complement thereof

(j) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein G at nucleotide position 2753 is substituted with A, or a complement thereof

(k) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein T at nucleotide position 2759 is substituted with C, or a complement thereof

(l) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:1, wherein G at nucleotide position 2829 is substituted with A, or a complement thereof

(m) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:7, wherein T at nucleotide position 190 is substituted with C, or a complement thereof;

(n) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:7, wherein C at nucleotide position 503 is substituted with T, or a complement thereof;

(o) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:7, wherein G at nucleotide position 927 is substituted with A, or a complement thereof;

(p) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:7, wherein C at nucleotide position 928 is substituted with T, or a complement thereof;

(q) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:7, wherein G at nucleotide position 1093 is substituted with A, or a complement thereof;

(r) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:7, wherein C at nucleotide position 1340 is substituted with T, or a complement thereof; and

(s) a DNA molecule comprising the nucleotides set forth in SEQ ID NO:7, wherein G at nucleotide position 1529 is substituted with T, or a complement thereof;

3. A polypeptide selected from the group consisting of:

(a) a protein molecule comprising the amino acids set forth in SEQ ID NO:7, wherein Cys at position 51 is substituted with Arg;

(b) a protein molecule comprising the amino acids set forth in SEQ ID NO:7, wherein Ala at position 155 is substituted with Val;

(c) a protein molecule comprising the amino acids set forth in SEQ ID NO:7, wherein Pro at position 297 is substituted with Ser;

(d) a protein molecule comprising the amino acids set forth in SEQ ID NO:7, wherein Asp at position 352 is substituted with Asn; and

(e) a protein molecule comprising the amino acids set forth in SEQ ID NO:7, wherein Ala at position 434 is substituted with Val.

4. A method for detecting an alteration in *AGT* wherein said alteration is associated with IDDM in a human, wherein said method comprises analyzing an *AGT* gene or an *AGT* gene expression product from a tissue of said human.

5. A method as claimed in claim 4 wherein the sequence of the *AGT* gene in said sample is compared with the sequence of one or more wild-type *AGT* gene sequences.

6. A method for determining whether a human subject has or is at risk for developing diabetes mellitus comprising the step of:

a) obtaining a sample from a subject, said sample comprising nucleic acid molecules containing AGT gene; and

b) detecting the presence or absence of a genetic polymorphism in the gene of said subject, wherein the presence of said genetic polymorphism identifies a subject that has or is at risk for developing diabetes.

7. A method for determining whether a human subject has or is at risk for developing diabetes mellitus comprising the step of:

a) obtaining a sample from a subject, said sample comprising nucleic acid molecules containing AGT gene; and

b) detecting the presence or absence of a genetic polymorphism in the gene of said subject, wherein the presence of said genetic polymorphism identifies a subject that has or is at risk for developing diabetes,

wherein said polymorphism is one of those specified in claim 2.

8. A method for preventing or treating diabetes in a subject which comprises administering to the subject a therapeutically effective amount of a compound that agonizes or antagonizes wild-type AGT, or agonizes or antagonizes mutant AGT, or agonizes or antagonizes an AGT receptor.

9. The method of claim 8, wherein said compound is selected from the group consisting of:

(a) a drug;

(b) an antisense molecule;

(c) a ribozyme;

(d) a triplex molecule;

(e) wild-type *AGT* nucleic acid;

(f) wild-type AGT protein;

(g) a protein which binds wild-type or mutant AGT protein:

(h) a peptidomimetic;

(i) a non-peptide, non-nucleic acid small molecule; and .

(j) an antibody.

10. A method for preventing or treating diabetes in a subject which comprises administering to the subject a therapeutically effective amount of a compound that agonizes or antagonizes wild-type AGT fragment(s), or agonizes orantagonizes mutant AGT, or agonizes or antagonizes a receptor for a fragment or fragments of AGT.

11. The method of claim 10, wherein said compound is selected from the group consisting of:

(a) a drug;

(b) an antisense molecule;

(c) a ribozyme;

(d) a triplex molecule;

(e) wild-type *AGT* nucleic acid;

(f) wild-type AGT protein;

(g) a protein which binds wild-type or mutant AGT protein:

(h) a peptidomimetic;

(i) a non-peptide, non-nucleic acid small molecule; and .

(j) an antibody.

12. A method for preventing or treating diabetes in a subject which comprises administering to the subject a therapeutically effective amount of a compound that agonizes or antagonizes a protease that cleaves AGT or any of its fragments.

13. The method of claim 12, wherein said compound is selected from the group consisting of:

(a) a drug;

(b) an antisense molecule;

(c) a ribozyme;

(d) a triplex molecule;

(e) wild-type *AGT* nucleic acid;

(f) wild-type AGT protein;

(g) a protein which binds wild-type or mutant AGT protein:

(h) a peptidomimetic;

(i) a non-peptide, non-nucleic acid small molecule; and .

(j) an antibody.

14. A non-human animal which carries an altered *AGT* allele in its genome, wherein said allele comprises one of the polymorphisms set forth in claim 2.

15. A cell line isolated from the non-human animal of claim 14.

16. A method of screening for drug candidates useful in treating diabetes resulting from a mutation in *AGT*, wherein said method involves mixing a mutant AGT in both the presence of a drug and the absence of said drug and measuring the level of the biological activity of the mutant AGT, wherein if the level of the biological activity is less in the presence of said drug than in the absence of said drug then said drug is a drug candidate for treating diabetes.

17. A method of screening for drug candidates useful in treating diabetes resulting from a mutation in *AGT*, wherein said method comprises treating an animal which is heterozygous or homozygous for *AGT* containing said mutation with a drug wherein if said animal does not develop diabetes then said drug is a drug candidate for treating diabetes.

18. A method for screening potential diabetes therapeutics which comprises: combining an AGT binding partner and a compound suspected of being an diabetes therapeutic and measuring the biological activity of the binding partner.

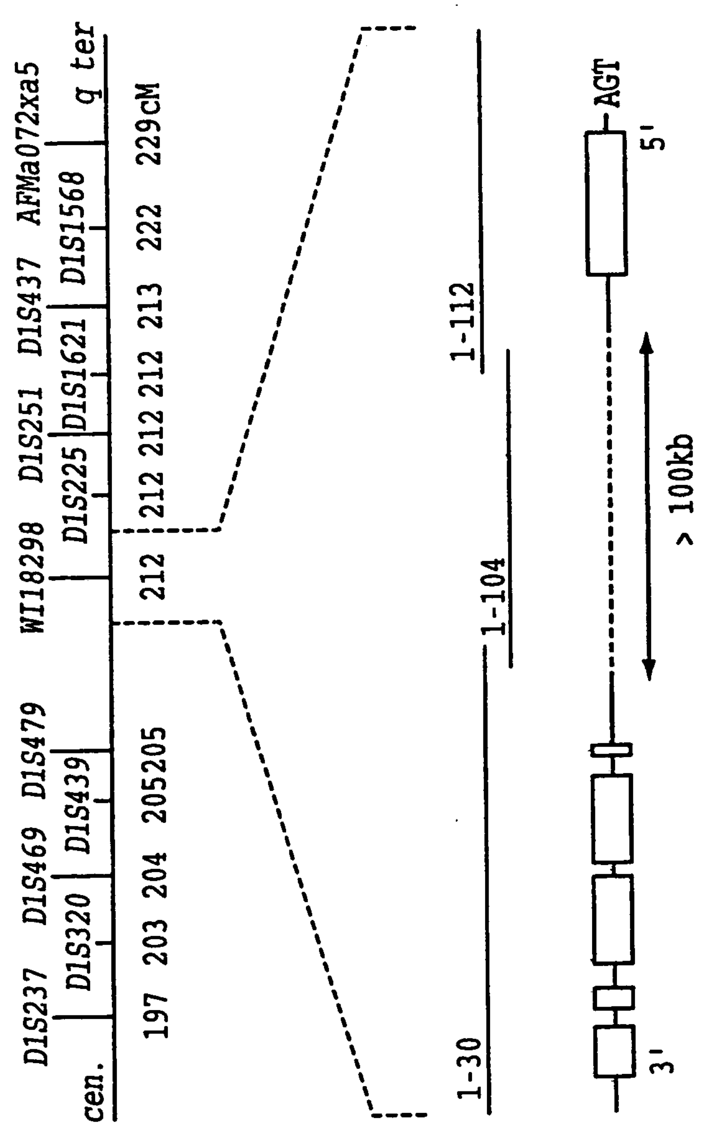
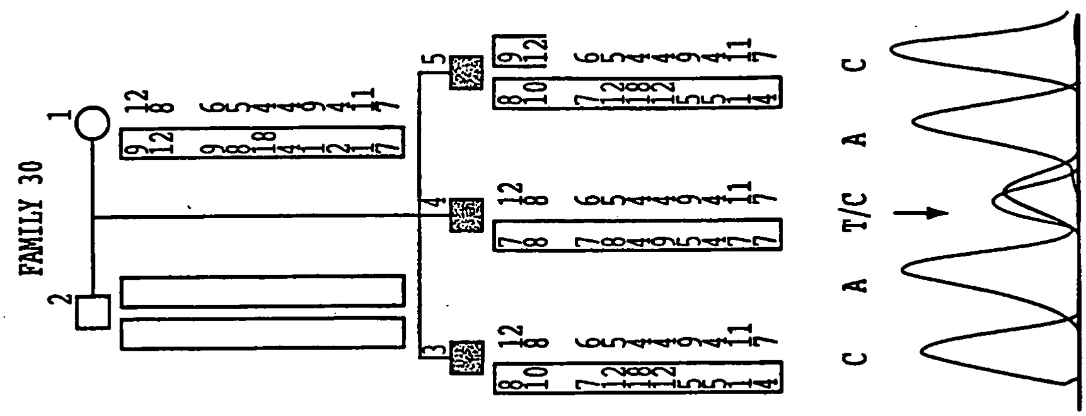


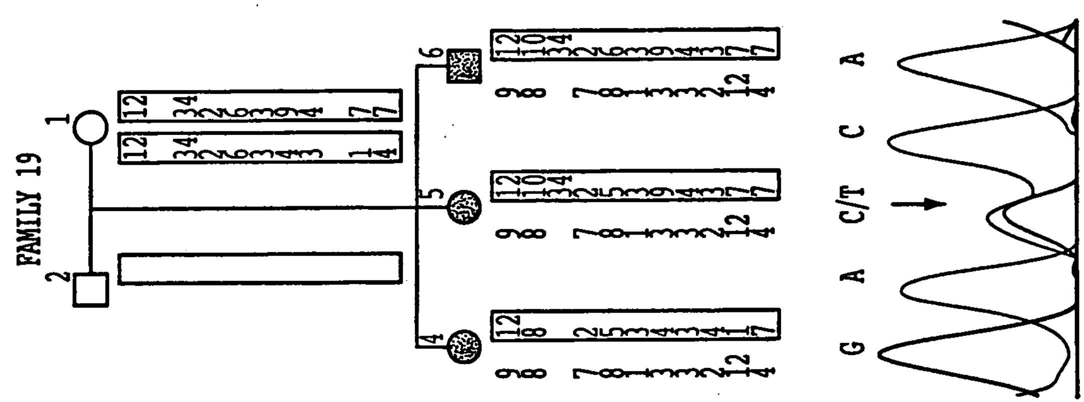
FIG. 1A

FIG. 1B

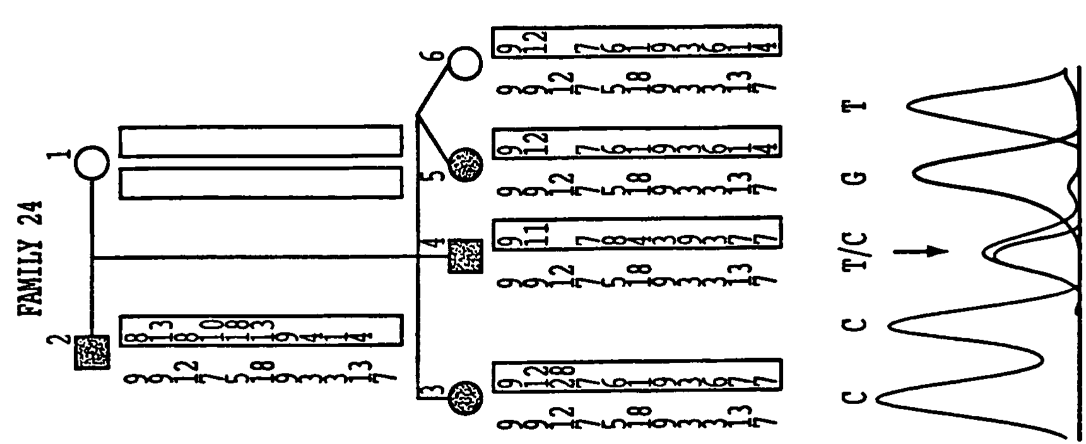
FIG. 1C



T286C alt1
FIG. 2C



C96T alt1
FIG. 2B



Cys18Arg
FIG. 2A

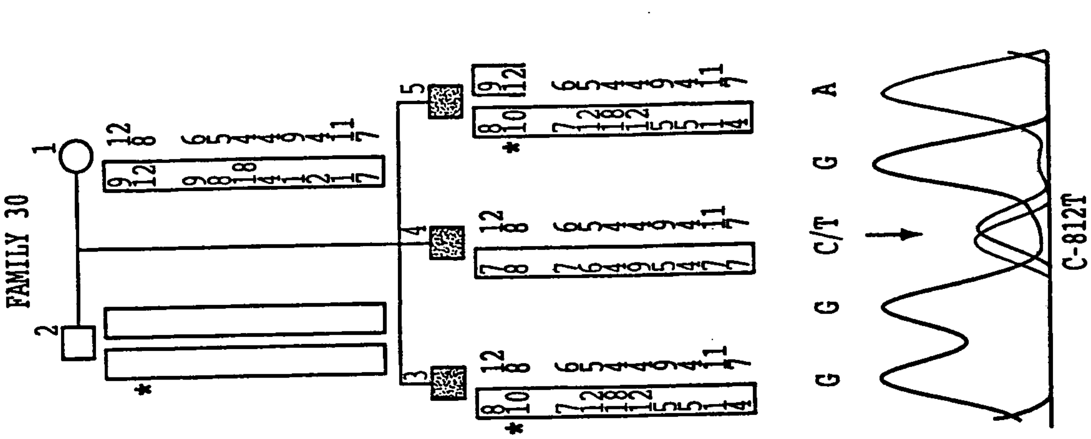


FIG. 2E

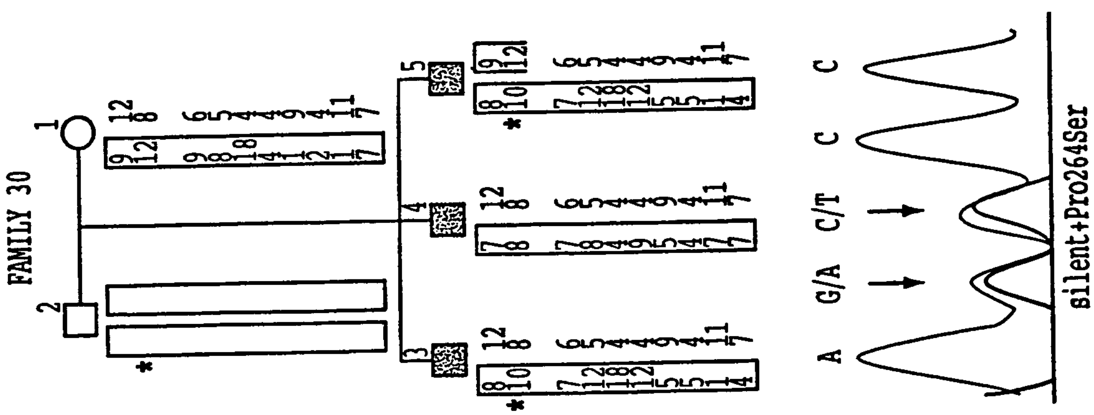
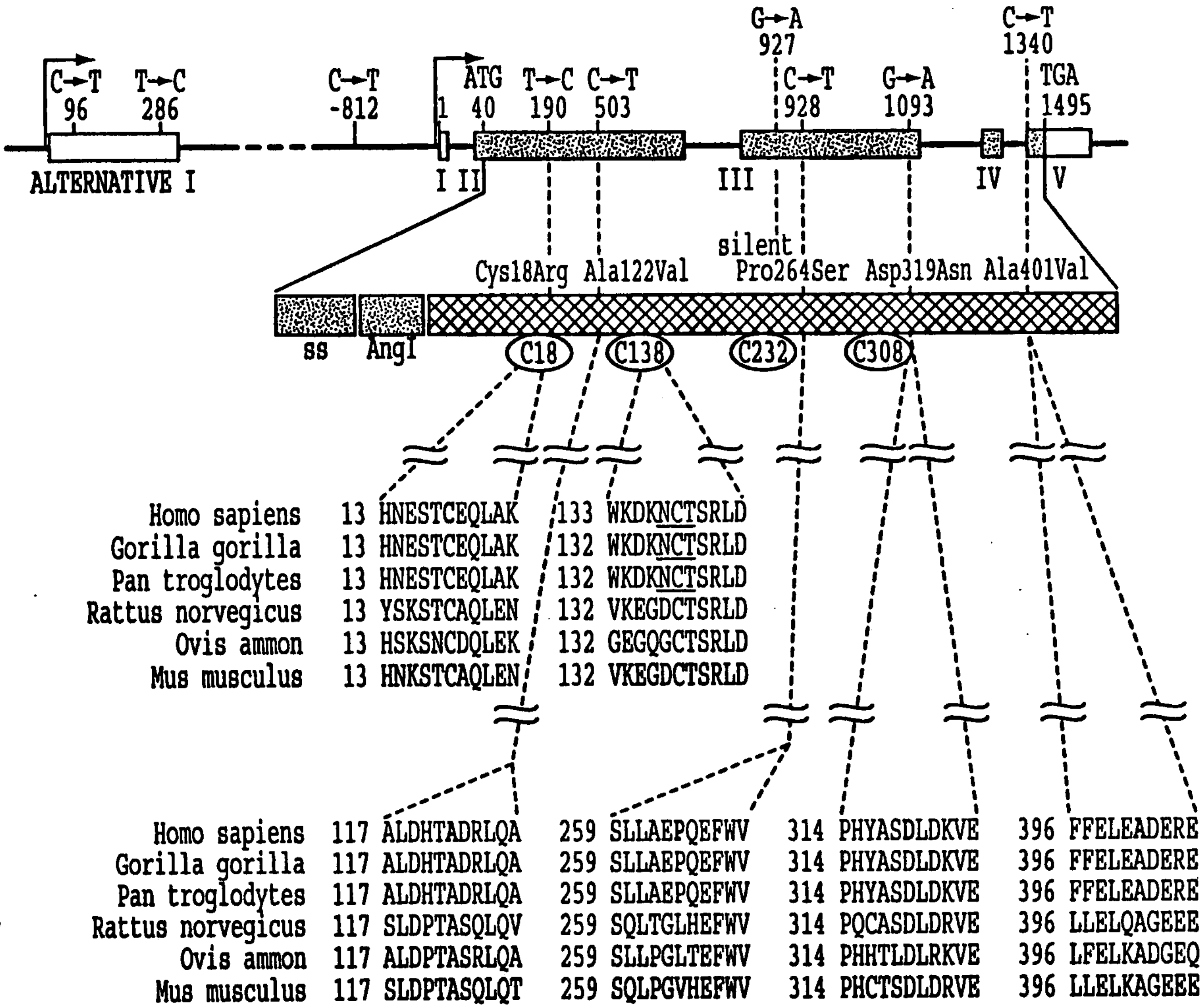


FIG. 2D

FIG. 3A

FIG. 3B

FIG. 3C



SEQUENCE LISTING

<110> McGrail, Maura
Russell, Deanna L.
Shattuck, Donna M.
Myriad Genetics, Inc.

<120> DIABETES GENE

<130> Diabetes Gene III

<140>
<141>

<150> US 60/174,700
<151> 2000-01-06

<150> US 60/135,423
<151> 1999-05-21

<160> 68

<170> PatentIn Ver. 2.0

<210> 1
<211> 5308
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (3012)..(3047)
<223> exon 1 of AGT gene

<400> 1
gccccagcca cttgacccaa cctggagctc acaaaacccc gtgttgtata aaagtaaggt 60

ttaagggtct agggctgtgt caaagtggct ggggcaaagc tacaaattaa caacatctca 120

gcaaagcaat tgtttaaagt acagctcttt ttcaaaatgg agtctcttat gtcttccctt 180

tctatataga cacagtaaca gtctgatctc tctttttttt ccctacatat cccaatagca 240

cctgcctgac taatacatca tgctctgctg actccatatg tggctggttt cctggatccg 300

attctgactg atggtatctg ttctcacgct gctaataaag acataccaga gactgggtaa 360

tttataaaga aaaagaggtt taatggactt atgtgttcca cgtggctggg gaggcctcac 420

aatcatggtg gaaggtgaag gaggagcaaa agcacatctt acatggtggc tggcaaaaga 480

gagaaagcat gttcagggga actccccttt ataaaaccat cagatctcat gagacttatg 540

cactatcaca agaacagcac cagaaagatc caccctcatg attcaattac ctcccactgg 600

gtccctcccg tgacacatgg gaattatggg agctacaatt ggagatttgg gtggggacac 660

agccaaacca tatcagatgg cttatttggt ttctatgtag aacctctgct tttcattcaa 720

cagtcttcat ttagccacag ataagctctg tccctaactt ccgctgatgg aatgtacaca 780

taagaaactt ccactgatgg aatgaacaca gaaggtgcct actgggaaga aaactggcct 840

gaatctgagc tgggtcaaat gtctgcagtc agtttgaatg gctgctcctt atgggaataa 900

tttacattct caataaaatt ctctagcaat tttctgattg attttaatga gctttaaagc 960

cttacgtaga agatccccca gctgatagtc agccttgggc atggattaag ggcttttaac 1020

caatcttgca acaagtttaa gcagatattc tttattgggt ccaatctaac caaaattatt 1080

ttcttatgtt ctccccagta acgtgtcatt attaagagaa gtttggcttg cttagaggcc 1140

aaatttagag ggtcctgaaa ttttattttc ttttacacca ctttccagca tgttacctga 1200

tcagttgttt attatctttg ctgttgaatg gagtgatcat tccaagggcc cgaggcagga 1260

ggcccaggca cagtggaaac tctcccaaag accaggatct ttgttttgtt ccctgacata 1320

tgctgagcac caggaatagt gaatgaatga aacaaattgt gaggctttaa agagccgaaa 1380

tatttaaaca ctgggcacaa ggttgttgct taatcagtgc tagatcctta cctccccctt 1440

gtgtccaggt cgacttgtta ctgcagttaa accacttgct gatcctcaaa caactagtta 1500

gtggcacagc caggcctagg accccagtct ctactgttcc aactaaccca ttcgcaggca 1560

ggagcacttt gaatggtctc ttattttaaa aaaattaaat taaaattgtc tatttattta 1620

gagacagagt cttactctgt agcccaggct cgagtgcagt ggtgcaatca tagctcactg 1680

taacctccat ctcctggcct caaaaagtgt ttgaattaca gatgcgaggc actgtacctg 1740

gcccgaatgt tctgttcaga caaagccacc tctaagtcgc tgtggggccc cagacaagtg 1800

atttttgagg agtccctatc tataggaaca aagtaattaa aaaaatgtat ttcagaattt 1860

acaggcccat gtgagatatg atttttttaa atgaagattt agagtaatgg gtaaaaaaga 1920

ggtatttgtg tgtttgttga ttgttcagtc agtgaatgta cagcttctgc ctcatatcca 1980

ggcaccatct cttcctgctc tttgttgtta aatgttccat tcctgggtaa tttcatgtct 2040

gccatcgtgg atatgccgtg gctccttgaa cctgcttgtg ttgaagcagg atcttccttc 2100

ctgtcccttc agtgccctaa taccatgtat ttaaggctgg acacatcacc actcccaacc 2160

tgcctcaccc actgcgtcac ttgtgatcac tggcttctgg cgactctcac caaggtctct 2220

gtcatgccct gttataacga ctacaaaagc aagtcttacc tataggaaaa taagaattat 2280

aacccttttta ctggtcatgt gaaacttacc atttgcaatt tgtacagcat aaacacagaa 2340

cagcacatct ttcaatgcct gcatcctgaa ggcattttgt ttgtgtcttt caatctggct 2400

gtgctattgt tggtgtttaa cagtctcccc agctacactg gaaacttcca gaaggcactt 2460

ttcacttgct tgtgtgtttt ccccagtgtc tattagaggc ctttgcacag ggtaggctct 2520

ttggagcagc tgaaggtcac acatcccatg agcgggcagc agggtcagaa gtggcccccg 2580

tgttgcctaa gcaagactct cccctgccct ctgccctctg cacctccggc ctgcatgtcc 2640

ctgtggcctc ttgggggtac atctcccggg gctgggtcag aaggcctggg tggttggcct 2700

caggctgtca cacacctagg gagatgctcc cgtttctggg aaccttggcc ccgactcctg 2760

caaacttcgg taaatgtgta actcgaccct gcaccggctc actctgttca gcagtgaaac 2820

tctgcatcga tcactaagac ttcctggaag aggtcccagc gtgagtgtcg cttctggcat 2880

ctgtccttct ggccagcctg tggtctggcc aagtgatgta accctcctct ccagcctgtg 2940

cacaggcagc ctgggaacag ctccatcccc acccctcagc tataaatagg gcatcgtgac 3000

ccggccaggg gaagaagctg ccgttgttct gggtactaca gcagaaggta agccgggggc 3060

cccctcagct ccttctcggc cttgtctctc tcagatgtaa ctgagctgtg ggctaggagg 3120

aaaaggcccg ggaggaggca cggtgatgac tgaaaaacct ctcccctctc ataagaccag 3180

tcatccggac gcgggctttc ccccactcgg tgcccacctg ggtcttaca ggaggagctg 3240

ctcctcctca gcaataggac aagatggtca ggtcttcctg ttccgctgag aaaagttagg 3300

gtcctcagga acggagcaga ctggtacagg aacagagtca tcatggccaa gagtccaccg 3360

ggtcctcttg ccatcaggag gaatagcagg gcttgtgcag gaattggggc tggagggaag 3420

ggccgggctc ggtcagtctc cagctgggat ccccagagtg gtcaccctac ccctccctcg 3480

agacagactg cctgactgtg tgtcatcagg ctggtcaccg tctccctgaa cctcgatttg 3540

ctcacctata aaatggaact aataacgatg cctgggctcc ctgtctcagg ggctctggta 3600

tagctgaaga gaactaatat aacatgaaag tgctttctaa gctttgggat aagctaaaag 3660

gcagattcca attttattcg agggcagcgt agattggtgc ttcagctcgt ggatgacaga 3720

gtcagggggc ctggttctga gtcctagttc tgtctcttcc cagctgtgtg acgttgaaca 3780

agccactgga cctctctgtt cctctgcaaa acagcatgaa ccaattcatt aactacttct 3840

ccaggatgca gtaggtccca gggactatcc taggaatgtg ggctgtatta gtaaacacaa 3900

cagcgggaac cctgttccgg ggctcacatt cacatcagag caaacagaca aagacgctgg 3960

acagaataag tgcataacta catggtacag agggttataa ggagggaaaa ggggagctgg 4020

atgagagagt tgagagtgcc cggtgtggtg gggaaagctg cagggtgaaa tactgcatca 4080

gggaaacctc agggaaggtg aggactatgg tgaggtcaga ggggttgata tgagaacagt 4140

gccctgcaaa tggcaggcac cacaggagca tgagccgtca tcttcacctt tagcattcag 4200

cccgggagaa gtagggagac atagaagggg caggtgctgg ccaagaggca ggggcaggag 4260

aggagaaggc ggaggggcac tcagggcgag ggtgtcaggc ccgccacccc agagcaccat 4320

tactcccagg acgcggctgc gtgcagacct ggaaccagcc tagggagcag ccgcagatca 4380

caactgagaa caaacgacag tctctgcctc aaaaatggcc catggaattg cgtctctgga 4440

gacgctgcct gagcaggagc agcacagtga gcgggctgca tcgaccagcg ccatccaaac 4500

cccgaacagt tggcgcttgt caggcaggac ttcccagcag tcggttccca caggtttccc 4560

ctgttgacct gatttgatgt gactgtctag attaggtgtg aactggtggc ttaggcttct 4620

ctgcacagaa aggcctgcaa gcagcagaga gagttttctg ttccattttt ccatgtcatg 4680

tggctcttcc tgagaacagc ggatggagtc aaatgcatgg ggagtggggt gagatggtag 4740

ctgaggtcag aatttggcat ttgaatgact gaagcagaac aaaacacacc aggtacttca 4800

gcagctgcac cgtgttgagg gcaggtgctg gttacgggtc tgggtgaggg aagccagctg 4860

ccaatgtaag aagaatgact gggtatgctt agatgaagca gaaaaatcta ggcatcaagg 4920

tggccttgag tcagtgatga cacgctacag ctccaaggaa gcctggccta gccctggggg 4980

gacagaaaag gccaagaagt gacgatattg cagtacaccc ccctccacaa gaaatgagtg 5040

agatgtggta caaaatgtta gaattgaatg aatcaataga ataaacgttc atcccttcaa 5100

tcaagaagag tcagatgaaa tgaattagca gggccagccc aagaacctct tctgggggtc 5160

tcagggtagc tttcatttgt agcagctgag gcggaaccag ctgtaaggcc tttgagagaa 5220

cgtggtgctg gacccgtgtc tagggcaggg gttctaaacc ctgcttacat atcagagtca 5280

cctgagaatt ttctattttt tttttttt 5308

<210> 2
<211> 1159
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(1009)
<223> exon 2 of AGT gene

<400> 2
aatccatttc ttcttttttt tgtagacatg aatggattta ttctgggcta aatggtgaca 60

gggaatattg agacaatgaa agatctggtt agatggcact taaaggtcag ttaataacca 120

cctttcaccc tttgcaaaat gatatttcag ggtatgcgga agcgagcacc ccagtctgag 180

atggctcctg ccggtgtgag cctgagggcc accatcctct gcctcctggc ctgggctggc 240

ctggctgcag gtgaccgggt gtacatacac cccttccacc tcgtcatcca caatgagagt 300

acctgtgagc agctggcaaa ggccaatgcc gggaagccca aagaccccac cttcatacct 360

gctccaattc aggccaagac atcccctgtg gatgaaaagg ccctacagga ccagctggtg 420

ctagtcgctg caaaacttga caccgaagac aagttgaagg ccgcaatggt cgggatgctg 480

gccaacttct tgggcttccg tatatatggc atgcacagtg agctatgggg cgtggtccat 540

ggggccaccg tcctctcccc aacggctgtc tttggcaccc tggcctctct ctatctggga 600

gccttggacc acacagctga caggctacag gcaatcctgg gtgttccttg gaaggacaag 660

aactgcacct cccggctgga tgcgcacaag gtcctgtctg ccctgcaggc tgtacagggc 720

ctgctagtgg cccagggcag ggctgatagc caggcccagc tgctgctgtc cacggtggtg 780

ggcgtgttca cagccccagg cctgcacctg aagcagccgt ttgtgcaggg cctggctctc 840

tataccccctg tggtcctccc acgctctctg gacttcacag aactggatgt tgctgctgag 900

aagattgaca ggttcatgca ggctgtgaca ggatggaaga ctggctgctc cctgacggga 960

gccagtgtgg acagcaccct ggctttcaac acctacgtcc acttccaagg taaggcaaac 1020

ctctctgctg gctctggccc taggacttag tatccaatgt gtagctgaga tcagccagtc 1080

aggccttgga gatgggcagg gggcagccct gcggacatac ctggtgacca cccttgagaa 1140

gtggggaagt ggcctctcc 1159

<210> 3
<211> 568
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(418)
<223> exon 3 of AGT gene

<400> 3
ccaggagaat gcagagtggc ctgggggggg ccaaggaagg aggctgaggc agggcgaggg 60

gcaggatctg ggcctttggt gtctgccagc cctcattcct gcccctgtct tgggtgactc 120

ttccctccct gtctcctgtc tggatttcag ggaagatgaa gggcttctcc ctgctggccg 180

agccccagga gttctgggtg gacaacagca cctcagtgtc tgttcccatg ctctctggca 240

tgggcacctt ccagcactgg agtgacatcc aggacaactt ctcggtgact caagtgccct 300

tcactgagag cgcctgcctg ctgctgatcc agcctcacta tgcctctgac ctggacaagg 360

tggagggtct cactttccag caaaactccc tcaactggat gaagaaactg tctccccggt 420

aggagcctcc cggtctcccc tggaatgtgg gagccacact gtcctgccca ggctgggggc 480

ggggtgggga gtagacacac ctgagctgag ccttgggtgc agagcagggc agggccgcgg 540

tggcacgggg ctgggcaggc ggcctgtg 568

<210> 4
<211> 445

<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(295)
<223> exon 4 of AGT gene

<400> 4
cccaaggggg aggatgactt agttgggtga tggggggagc aaacatccct gtcgtcaggg 60

ttgggtgcaa ggagcataag cctgcctggc ctctgggaga gccctcactg tgtggcctgg 120

agccttccta actgtgcatc atctccccag gaccatccac ctgaccatgc cccaactggt 180

gctgcaagga tcttatgacc tgcaggacct gctcgcccag gctgagctgc ccgccattct 240

gcacaccgag ctgaacctgc aaaaattgag caatgaccgc atcagggtgg gggaggtatg 300

tgtgagcctg tgtctgtgcc tgacctgggt tccaagtgtg cacagggtgg gaggcatgga 360

tgtaagggac acagaggagg ctatgggtgg ggccagcagg gcaagaggga gcggagagta 420

gggccaaagg tgggagagaa gtagc 445

<210> 5
<211> 1091
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(941)
<223> exon 5 of AGT gene

<400> 5
actgtgcatc ctcctggcag gctggaaaat gtccccctcc aggacagtgc acagcacaga 60

ggtcctgagc ccaccccggc cctctagccc tcagcaccct gggtcaccca gtgcgccctc 120

agaatgatcc tgatgtctgc tgctttgcag gtgctgaaca gcattttttt tgagcttgaa 180

gcggatgaga gagagcccac agagtctacc caacagctta acaagcctga ggtcttggag 240

gtgaccctga accgcccatt cctgtttgct gtgtatgatc aaagcgccac tgccctgcac 300

ttcctgggcc gcgtggccaa cccgctgagc acagcatgag gccagggccc cagaacacag 360

tgcctggcaa ggcctctgcc cctggccttt gaggcaaagg ccagcagcag ataacaaccc 420

cggacaaatc agcgatgtgt cacccccagt ctcccacctt ttcttctaat gagtcgactt 480

tgagctggaa agcagccgtt tctccttggt ctaagtgtgc tgcatggagt gagcagtaga 540

agcctgcagc ggcacaaatg cacctcccag tttgctgggt ttattttaga gaatgggggt 600

ggggaggcaa gaaccagtgt ttagcgcggg actactgttc caaaaagaat tccaaccgac 660

cagcttgttt gtgaaacaaa aaagtgttcc cttttcaagt tgagaacaaa aattgggttt 720

```
taaaattaaa gtatacattt ttgcattgcc ttcggtttgt atttagtgtc ttgaatgtaa 780

gaacatgacc tccgtgtagt gtctgtaata ccttagtttt ttccacagat gcttgtgatt 840

tttgaacaat acgtgaaaga tgcaagcacc tgaatttctg tttgaatgcg gaaccatagc 900

tggttatttc tcccttgtgt tagtaataaa cgtcttgcca caataagcct ccaaaaattt 960

tatctttcat ttagcagcca aacagatgta tacaattcag cagatagact gtgcaaacga 1020

aagtgctttc ctggactttg gatggaattt ccatgggagg tctgagccag tacttagcag 1080

tcctttgaag t                                                      1091


<210> 6
<211> 2099
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (40)..(1494)

<220>
<221> sig_peptide
<222> (40)..(138)

<400> 6
aagaagctgc cgttgttctg ggtactacag cagaagggt atg cgg aag cga gca     54
                                           Met Arg Lys Arg Ala
                                            1               5

ccc cag tct gag atg gct cct gcc ggt gtg agc ctg agg gcc acc atc    102
Pro Gln Ser Glu Met Ala Pro Ala Gly Val Ser Leu Arg Ala Thr Ile
                10                  15                  20

ctc tgc ctc ctg gcc tgg gct ggc ctg gct gca ggt gac cgg gtg tac    150
Leu Cys Leu Leu Ala Trp Ala Gly Leu Ala Ala Gly Asp Arg Val Tyr
            25                  30                  35

ata cac ccc ttc cac ctc gtc atc cac aat gag agt acc tgt gag cag    198
Ile His Pro Phe His Leu Val Ile His Asn Glu Ser Thr Cys Glu Gln
        40                  45                  50

ctg gca aag gcc aat gcc ggg aag ccc aaa gac ccc acc ttc ata cct    246
Leu Ala Lys Ala Asn Ala Gly Lys Pro Lys Asp Pro Thr Phe Ile Pro
    55                  60                  65

gct cca att cag gcc aag aca tcc cct gtg gat gaa aag gcc cta cag    294
Ala Pro Ile Gln Ala Lys Thr Ser Pro Val Asp Glu Lys Ala Leu Gln
70                  75                  80                  85

gac cag ctg gtg cta gtc gct gca aaa ctt gac acc gaa gac aag ttg    342
Asp Gln Leu Val Leu Val Ala Ala Lys Leu Asp Thr Glu Asp Lys Leu
                90                  95                  100

aag gcc gca atg gtc ggg atg ctg gcc aac ttc ttg ggc ttc cgt ata    390
Lys Ala Ala Met Val Gly Met Leu Ala Asn Phe Leu Gly Phe Arg Ile
            105                 110                 115
```

```
tat ggc atg cac agt gag cta tgg ggc gtg gtc cat ggg gcc acc gtc    438
Tyr Gly Met His Ser Glu Leu Trp Gly Val Val His Gly Ala Thr Val
        120                 125                 130

ctc tcc cca acg gct gtc ttt ggc acc ctg gcc tct ctc tat ctg gga    486
Leu Ser Pro Thr Ala Val Phe Gly Thr Leu Ala Ser Leu Tyr Leu Gly
    135                 140                 145

gcc ttg gac cac aca gct gac agg cta cag gca atc ctg ggt gtt cct    534
Ala Leu Asp His Thr Ala Asp Arg Leu Gln Ala Ile Leu Gly Val Pro
150                 155                 160                 165

tgg aag gac aag aac tgc acc tcc cgg ctg gat gcg cac aag gtc ctg    582
Trp Lys Asp Lys Asn Cys Thr Ser Arg Leu Asp Ala His Lys Val Leu
                170                 175                 180

tct gcc ctg cag gct gta cag ggc ctg cta gtg gcc cag ggc agg gct    630
Ser Ala Leu Gln Ala Val Gln Gly Leu Leu Val Ala Gln Gly Arg Ala
            185                 190                 195

gat agc cag gcc cag ctg ctg ctg tcc acg gtg gtg ggc gtg ttc aca    678
Asp Ser Gln Ala Gln Leu Leu Leu Ser Thr Val Val Gly Val Phe Thr
        200                 205                 210

gcc cca ggc ctg cac ctg aag cag ccg ttt gtg cag ggc ctg gct ctc    726
Ala Pro Gly Leu His Leu Lys Gln Pro Phe Val Gln Gly Leu Ala Leu
    215                 220                 225

tat acc cct gtg gtc ctc cca cgc tct ctg gac ttc aca gaa ctg gat    774
Tyr Thr Pro Val Val Leu Pro Arg Ser Leu Asp Phe Thr Glu Leu Asp
230                 235                 240                 245

gtt gct gct gag aag att gac agg ttc atg cag gct gtg aca gga tgg    822
Val Ala Ala Glu Lys Ile Asp Arg Phe Met Gln Ala Val Thr Gly Trp
                250                 255                 260

aag act ggc tgc tcc ctg acg gga gcc agt gtg gac agc acc ctg gct    870
Lys Thr Gly Cys Ser Leu Thr Gly Ala Ser Val Asp Ser Thr Leu Ala
            265                 270                 275

ttc aac acc tac gtc cac ttc caa ggg aag atg aag ggc ttc tcc ctg    918
Phe Asn Thr Tyr Val His Phe Gln Gly Lys Met Lys Gly Phe Ser Leu
        280                 285                 290

ctg gcc gag ccc cag gag ttc tgg gtg gac aac agc acc tca gtg tct    966
Leu Ala Glu Pro Gln Glu Phe Trp Val Asp Asn Ser Thr Ser Val Ser
    295                 300                 305

gtt ccc atg ctc tct ggc atg ggc acc ttc cag cac tgg agt gac atc    1014
Val Pro Met Leu Ser Gly Met Gly Thr Phe Gln His Trp Ser Asp Ile
310                 315                 320                 325

cag gac aac ttc tcg gtg act caa gtg ccc ttc act gag agc gcc tgc    1062
Gln Asp Asn Phe Ser Val Thr Gln Val Pro Phe Thr Glu Ser Ala Cys
                330                 335                 340

ctg ctg ctg atc cag cct cac tat gcc tct gac ctg gac aag gtg gag    1110
Leu Leu Leu Ile Gln Pro His Tyr Ala Ser Asp Leu Asp Lys Val Glu
            345                 350                 355
```

```
ggt ctc act ttc cag caa aac tcc ctc aac tgg atg aag aaa ctg tct    1158
Gly Leu Thr Phe Gln Gln Asn Ser Leu Asn Trp Met Lys Lys Leu Ser
        360                 365                 370

ccc cgg acc atc cac ctg acc atg ccc caa ctg gtg ctg caa gga tct    1206
Pro Arg Thr Ile His Leu Thr Met Pro Gln Leu Val Leu Gln Gly Ser
    375                 380                 385

tat gac ctg cag gac ctg ctc gcc cag gct gag ctg ccc gcc att ctg    1254
Tyr Asp Leu Gln Asp Leu Leu Ala Gln Ala Glu Leu Pro Ala Ile Leu
390                 395                 400                 405

cac acc gag ctg aac ctg caa aaa ttg agc aat gac cgc atc agg gtg    1302
His Thr Glu Leu Asn Leu Gln Lys Leu Ser Asn Asp Arg Ile Arg Val
                410                 415                 420

ggg gag gtg ctg aac agc att ttt ttt gag ctt gaa gcg gat gag aga    1350
Gly Glu Val Leu Asn Ser Ile Phe Phe Glu Leu Glu Ala Asp Glu Arg
            425                 430                 435

gag ccc aca gag tct acc caa cag ctt aac aag cct gag gtc ttg gag    1398
Glu Pro Thr Glu Ser Thr Gln Gln Leu Asn Lys Pro Glu Val Leu Glu
        440                 445                 450

gtg acc ctg aac cgc cca ttc ctg ttt gct gtg tat gat caa agc gcc    1446
Val Thr Leu Asn Arg Pro Phe Leu Phe Ala Val Tyr Asp Gln Ser Ala
    455                 460                 465

act gcc ctg cac ttc ctg ggc cgc gtg gcc aac ccg ctg agc aca gca    1494
Thr Ala Leu His Phe Leu Gly Arg Val Ala Asn Pro Leu Ser Thr Ala
470                 475                 480                 485

tgaggccagg gccccagaac acagtgcctg gcaaggcctc tgcccctggc ctttgaggca 1554

aaggccagca gcagataaca accccggaca aatcagcgat gtgtcacccc cagtctccca 1614

ccttttcttc taatgagtcg actttgagct ggaaagcagc cgtttctcct tggtctaagt 1674

gtgctgcatg gagtgagcag tagaagcctg cagcggcaca aatgcacctc ccagtttgct 1734

gggtttattt tagagaatgg gggtggggag gcaagaacca gtgtttagcg cgggactact 1794

gttccaaaaa gaattccaac cgaccagctt gtttgtgaaa caaaaaagtg ttcccttttc 1854

aagttgagaa caaaaattgg gttttaaaat taaagtatac atttttgcat tgccttcggt 1914

ttgtatttag tgtcttgaat gtaagaacat gacctccgtg tagtgtctgt aatacettag 1974

ttttttccac agatgcttgt gatttttgaa caatacgtga aagatgcaag cacctgaatt 2034

tctgtttgaa tgcggaacca tagctggtta tttctccctt gtgttagtaa taaacgtctt 2094

gccac                                                             2099
```

```
<210> 7
<211> 485
<212> PRT
<213> Homo sapiens
```

<400> 7

Met Arg Lys Arg Ala Pro Gln Ser Glu Met Ala Pro Ala Gly Val Ser
1 5 10 15

Leu Arg Ala Thr Ile Leu Cys Leu Leu Ala Trp Ala Gly Leu Ala Ala
20 25 30

Gly Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His Asn Glu
35 40 45

Ser Thr Cys Glu Gln Leu Ala Lys Ala Asn Ala Gly Lys Pro Lys Asp
50 55 60

Pro Thr Phe Ile Pro Ala Pro Ile Gln Ala Lys Thr Ser Pro Val Asp
65 70 75 80

Glu Lys Ala Leu Gln Asp Gln Leu Val Leu Val Ala Ala Lys Leu Asp
85 90 95

Thr Glu Asp Lys Leu Lys Ala Ala Met Val Gly Met Leu Ala Asn Phe
100 105 110

Leu Gly Phe Arg Ile Tyr Gly Met His Ser Glu Leu Trp Gly Val Val
115 120 125

His Gly Ala Thr Val Leu Ser Pro Thr Ala Val Phe Gly Thr Leu Ala
130 135 140

Ser Leu Tyr Leu Gly Ala Leu Asp His Thr Ala Asp Arg Leu Gln Ala
145 150 155 160

Ile Leu Gly Val Pro Trp Lys Asp Lys Asn Cys Thr Ser Arg Leu Asp
165 170 175

Ala His Lys Val Leu Ser Ala Leu Gln Ala Val Gln Gly Leu Leu Val
180 185 190

Ala Gln Gly Arg Ala Asp Ser Gln Ala Gln Leu Leu Leu Ser Thr Val
195 200 205

Val Gly Val Phe Thr Ala Pro Gly Leu His Leu Lys Gln Pro Phe Val
210 215 220

Gln Gly Leu Ala Leu Tyr Thr Pro Val Val Leu Pro Arg Ser Leu Asp
225 230 235 240

Phe Thr Glu Leu Asp Val Ala Ala Glu Lys Ile Asp Arg Phe Met Gln
245 250 255

Ala Val Thr Gly Trp Lys Thr Gly Cys Ser Leu Thr Gly Ala Ser Val
260 265 270

Asp Ser Thr Leu Ala Phe Asn Thr Tyr Val His Phe Gln Gly Lys Met
275 280 285

Lys Gly Phe Ser Leu Leu Ala Glu Pro Gln Glu Phe Trp Val Asp Asn
290 295 300

Ser Thr Ser Val Ser Val Pro Met Leu Ser Gly Met Gly Thr Phe Gln
305 310 315 320

```
His Trp Ser Asp Ile Gln Asp Asn Phe Ser Val Thr Gln Val Pro Phe
                325                 330                 335

Thr Glu Ser Ala Cys Leu Leu Leu Ile Gln Pro His Tyr Ala Ser Asp
            340                 345                 350

Leu Asp Lys Val Glu Gly Leu Thr Phe Gln Gln Asn Ser Leu Asn Trp
        355                 360                 365

Met Lys Lys Leu Ser Pro Arg Thr Ile His Leu Thr Met Pro Gln Leu
    370                 375                 380

Val Leu Gln Gly Ser Tyr Asp Leu Gln Asp Leu Leu Ala Gln Ala Glu
385                 390                 395                 400

Leu Pro Ala Ile Leu His Thr Glu Leu Asn Leu Gln Lys Leu Ser Asn
                405                 410                 415

Asp Arg Ile Arg Val Gly Glu Val Leu Asn Ser Ile Phe Phe Glu Leu
            420                 425                 430

Glu Ala Asp Glu Arg Glu Pro Thr Glu Ser Thr Gln Gln Leu Asn Lys
        435                 440                 445

Pro Glu Val Leu Glu Val Thr Leu Asn Arg Pro Phe Leu Phe Ala Val
    450                 455                 460

Tyr Asp Gln Ser Ala Thr Ala Leu His Phe Leu Gly Arg Val Ala Asn
465                 470                 475                 480

Pro Leu Ser Thr Ala
                485
```

<210> 8
<211> 681
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(531)
<223> altenate exon 1 of AGT gene

<400> 8
agggcgggga aggggggagg aaggggggact gagactctcc tgtttctata tgctatgatg 60

ggatttgacc tgcaattgaa cctttcctca aacacaaggc acaaacctgc cccagcccat 120

catcctccca cagaggcact gtgaatgtat aaagaaaggc gatgctaaga aacgtgctgc 180

aggggaggct ggcacattct gctccttagc taaccgcaca ggcccatggg tccacacaca 240

tcagaccatg gctctctgtg gtctatcttt gccattactt taaccaggct gtttctcatg 300

cgctcatgga gggtgactga agatgccacc ccgaggtgca ccctcagcct cgctggacac 360

ttacggctcg cattctccag ccggaccagg cagttgagga agtcatcgaa gtccagctgg 420

agctcctcat ccgcatacct gagcacaatc agctgcagga ggtggctgct cagctgaaag 480

cctgtgggga caaaggagga gacccaggac tcgctgtatc cgagccccga ggtgagtctg 540

cacctttgag ggagctccca ggcagctgag gagagggctc agaagaggaa gccactgttt 600

cactcctcaa agctggggtc cccagaccca gtgggactgc tgcccttggt gtgaatgcag 660

cgtgagagtg tgaatctgtg g 681

<210> 9
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:M13 forward primer

<400> 9
gttttcccag tcacgacg 18

<210> 10
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:M13 reverse primer

<400> 10
aggaaacagc tatgaccat 19

<210> 11
<211> 20
<212> DNA
<213> Homo sapiens

<400> 11
cctcgaagtg gcctatggaa 20

<210> 12
<211> 20
<212> DNA
<213> Homo sapiens

<400> 12
gcgagactct atgggtgatg 20

<210> 13
<211> 20
<212> DNA
<213> Homo sapiens

<400> 13
gactgagact ctcctgtttc 20

<210> 14
<211> 19
<212> DNA
<213> Homo sapiens

<400> 14
cggggtggca tcttcagtc 19

<210> 15
<211> 20
<212> DNA
<213> Homo sapiens

<400> 15
tggcacattc tgctccttag 20

<210> 16
<211> 20
<212> DNA
<213> Homo sapiens

<400> 16
atacagcgag tcctgggtct 20

<210> 17
<211> 20
<212> DNA
<213> Homo sapiens

<400> 17
gaccaggcag ttgaggaagt 20

<210> 18
<211> 20
<212> DNA
<213> Homo sapiens

<400> 18
gattcacact ctcacgctgc 20

<210> 19
<211> 20
<212> DNA
<213> Homo sapiens

<400> 19
ccactttcca gcatgttacc 20

<210> 20
<211> 20

<212> DNA
<213> Homo sapiens

<400> 20
ctgctattcc tcctgatggc 20

<210> 21
<211> 20
<212> DNA
<213> Homo sapiens

<400> 21
acaggcccat gtgagatatg 20

<210> 22
<211> 20
<212> DNA
<213> Homo sapiens

<400> 22
tggtattagg gcactgaagg 20

<210> 23
<211> 20
<212> DNA
<213> Homo sapiens

<400> 23
tgtgttgaag caggatcttc 20

<210> 24
<211> 20
<212> DNA
<213> Homo sapiens

<400> 24
gatgcaggca ttgaaagatg 20

<210> 25
<211> 21
<212> DNA
<213> Homo sapiens

<400> 25
gtgtttaaca gtctccccag c 21

<210> 26
<211> 20
<212> DNA
<213> Homo sapiens

<400> 26
ctaggtgtgt gacagcctga 20

<210> 27
<211> 19
<212> DNA
<213> Homo sapiens

<400> 27
catgtccctg tggcctctt 19

<210> 28
<211> 18
<212> DNA
<213> Homo sapiens

<400> 28
tgaggggtgg ggatggag 18

<210> 29
<211> 20
<212> DNA
<213> Homo sapiens

<400> 29
ctggtcatgt gaaacttacc 20

<210> 30
<211> 20
<212> DNA
<213> Homo sapiens

<400> 30
accttcagct gctccaaaga 20

<210> 31
<211> 20
<212> DNA
<213> Homo sapiens

<400> 31
ccactttcca gcatgttacc 20

<210> 32
<211> 20
<212> DNA
<213> Homo sapiens

<400> 32
ctgctattcc tcctgatggc 20

<210> 33
<211> 18
<212> DNA
<213> Homo sapiens

<400> 33
aaccctcctc tccagcct 18

<210> 34
<211> 18
<212> DNA
<213> Homo sapiens

<400> 34
tcatcaccgt gcctcctc 18

<210> 35
<211> 20
<212> DNA
<213> Homo sapiens

<400> 35
tacttggact ttgggctgag 20

<210> 36
<211> 21
<212> DNA
<213> Homo sapiens

<400> 36
ttgagcaagg cactttgttt c 21

<210> 37
<211> 21
<212> DNA
<213> Homo sapiens

<400> 37
ggctaaatgg tgacagggaa t 21

<210> 38
<211> 20
<212> DNA
<213> Homo sapiens

<400> 38
tagggccttt tcatccacag 20

<210> 39
<211> 20
<212> DNA
<213> Homo sapiens

<400> 39
aatgccggga agcccaaaga 20

<210> 40
<211> 20

<212> DNA
<213> Homo sapiens

<400> 40
tccaaggctc ccagatagag 20

<210> 41
<211> 20
<212> DNA
<213> Homo sapiens

<400> 41
ctctccccaa cggctgtctt 20

<210> 42
<211> 20
<212> DNA
<213> Homo sapiens

<400> 42
ctgcacaaac ggctgcttca 20

<210> 43
<211> 20
<212> DNA
<213> Homo sapiens

<400> 43
ctctatctgg gagccttgga 20

<210> 44
<211> 20
<212> DNA
<213> Homo sapiens

<400> 44
cacagcctgc atgaacctgt 20

<210> 45
<211> 20
<212> DNA
<213> Homo sapiens

<400> 45
tgaagcagcc gtttgtgcag 20

<210> 46
<211> 19
<212> DNA
<213> Homo sapiens

<400> 46
tccccacttc tcaagggtg 19

<210> 47
<211> 20
<212> DNA
<213> Homo sapiens

<400> 47
acaggttcat gcaggctgtg 20

<210> 48
<211> 19
<212> DNA
<213> Homo sapiens

<400> 48
tccccacttc tcaagggtg 19

<210> 49
<211> 21
<212> DNA
<213> Homo sapiens

<400> 49
tgcctcagtg aactcaaatg g 21

<210> 50
<211> 20
<212> DNA
<213> Homo sapiens

<400> 50
ctccccctcc aaaactacca 20

<210> 51
<211> 19
<212> DNA
<213> Homo sapiens

<400> 51
caggagaatg cagagtggc 19

<210> 52
<211> 19
<212> DNA
<213> Homo sapiens

<400> 52
gcaggcgctc tcagtgaag 19

<210> 53
<211> 20
<212> DNA
<213> Homo sapiens

<400> 53
agcactggag tgacatccag 20

<210> 54
<211> 20
<212> DNA
<213> Homo sapiens

<400> 54
gatggaggac tggtagacag 20

<210> 55
<211> 20
<212> DNA
<213> Homo sapiens

<400> 55
aagggggagg atgacttagt 20

<210> 56
<211> 21
<212> DNA
<213> Homo sapiens

<400> 56
gaaattccat ccaaagtcca g 21

<210> 57
<211> 20
<212> DNA
<213> Homo sapiens

<400> 57
gtgcaaggag cataagcctg 20

<210> 58
<211> 19
<212> DNA
<213> Homo sapiens

<400> 58
ctactctccg ctccctctt 19

<210> 59
<211> 20
<212> DNA
<213> Homo sapiens

<400> 59
aagggggagg atgacttagt 20

<210> 60
<211> 21

<212> DNA
<213> Homo sapiens

<400> 60
gaaattccat ccaaagtcca g 21

<210> 61
<211> 17
<212> DNA
<213> Homo sapiens

<400> 61
tagccctcag caccctg 17

<210> 62
<211> 19
<212> DNA
<213> Homo sapiens

<400> 62
tgtccggggt tgttatctg 19

<210> 63
<211> 20
<212> DNA
<213> Homo sapiens

<400> 63
agaacacagt gcctggcaag 20

<210> 64
<211> 20
<212> DNA
<213> Homo sapiens

<400> 64
ttcacaaaca agctggtcgg 20

<210> 65
<211> 20
<212> DNA
<213> Homo sapiens

<400> 65
agtgtttagc gcgggactac 20

<210> 66
<211> 20
<212> DNA
<213> Homo sapiens

<400> 66
tttggaggct tattgtggca 20

<210> 67
<211> 20
<212> DNA
<213> Homo sapiens

<400> 67
tgaaagatgc aagcacctga 20

<210> 68
<211> 20
<212> DNA
<213> Homo sapiens

<400> 68
gaaagcactt tcgtttgcac 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/13327

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 91.1, 91.31, 455, 375; 514/2, 4, 44; 536/23.1, 23.4, 23.5, 23.51, 24.3, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, WEST, Sequence searches: amino acid and nucleotide data bases (i.e. no size limits and oligo search).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,589,584 A (LALOUEL et al.) 31 December 1996, entire text, claims 1-3.	1-7
Y, A	O'BYRNE et al. Genetics of Hypertension. Therapeutic Implications. Drugs. August 1998, Vol. 56, No. 2, pages 203-214, especially pages 208-209.	1-7
Y	HIXSON et al. Detection and characterization of new mutations in the human angiotensinogen gene (AGT). Hum. Genet. 1995, Vol. 96, pages 110-112, see entire document.	1-7

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search 10 AUGUST 2000	Date of mailing of the international search report 02 OCT 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer D. Lawrence for JANE ZARA Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No. PCT/US00/13327

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OHNO et al. Association analyses of the polymorphisms of angiotension-converting enzyme and angiotensionogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. Metabolism Clin. and Expl. 1996, Vol. 45, No. 2, pages 218-222, especially pages 218 and 219.	1-7
X	WO 97/18312 A1 (VIMRX HOLDINGS LTD.) 22 May 1997, pages 4-15 and especially page 19.	8-11 and 16
Y,P	US 5,916,907 A (BIRD) 29 June 1999, col. 1, lines 17-55.	12,13
X - Y	WO 97/28684 A1 (ISHIKAWA et al.) 14 August 1997, abstract.	8-11, 14-18 ------ 8-11, 14-18
X	WO 95/19568 A1 (RATH, M.) 20 July 1995, abstract, page 76, claim 5.	8-11 and 16
X - Y	WO 98/58952 A1 (PROTEUS MOLECULAR DESIGN LIMITED) 30 December 1998, see entire document.	8-11, 16 ------ 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/13327

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C12Q 1/68; C12P 21/06, 19/34; A01N 43/04, 37/18; A61K 38/00, 38/28; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 69.1, 91.1, 91.31, 455, 375; 514/2, 4, 44; 536/23.1, 23.4, 23.5, 23.51, 24.3, 24.5

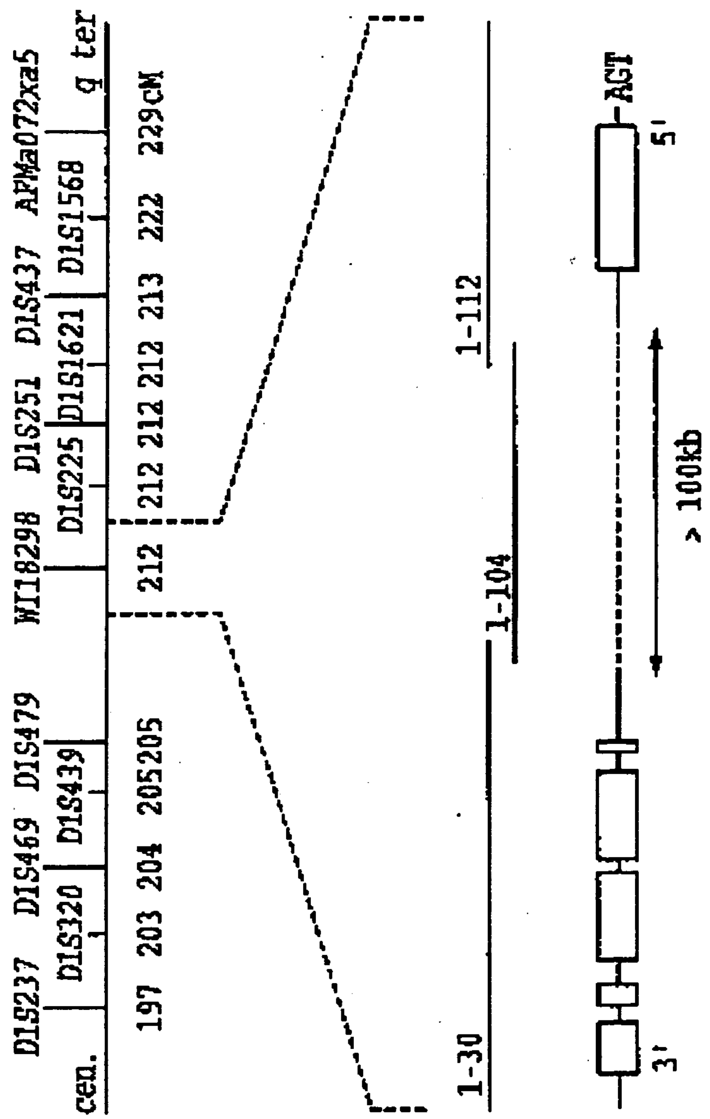


FIG. 1A

FIG. 1B

FIG. 1C

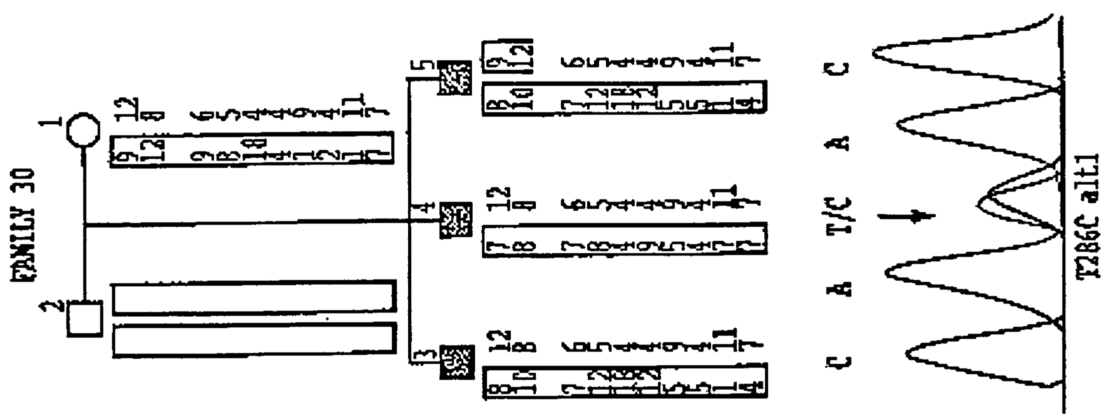


FIG. 2C

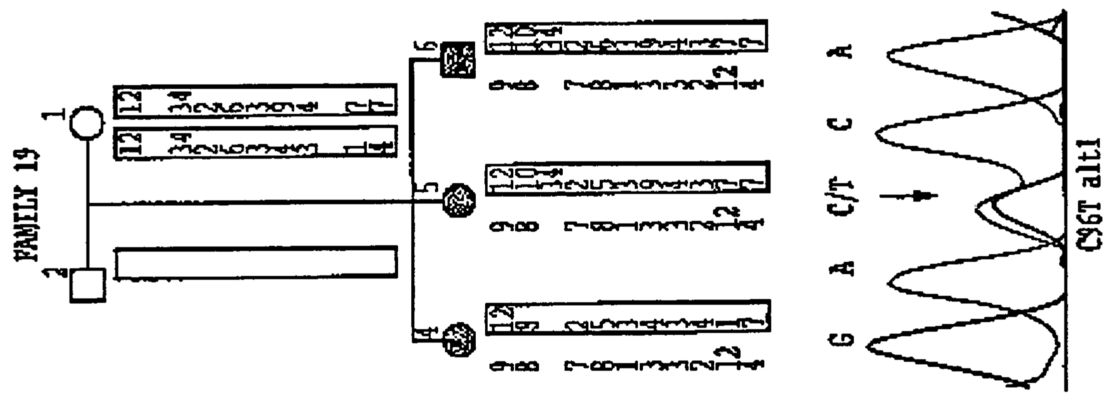


FIG. 2B

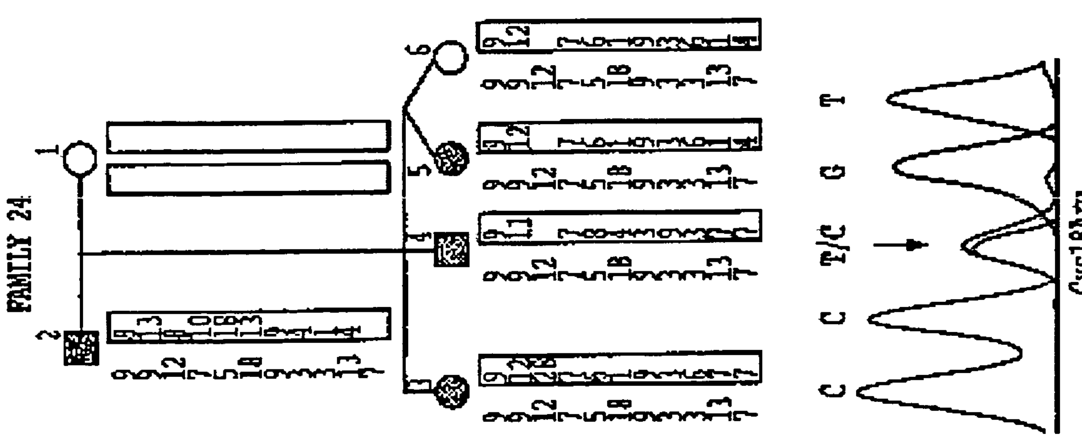


FIG. 2A

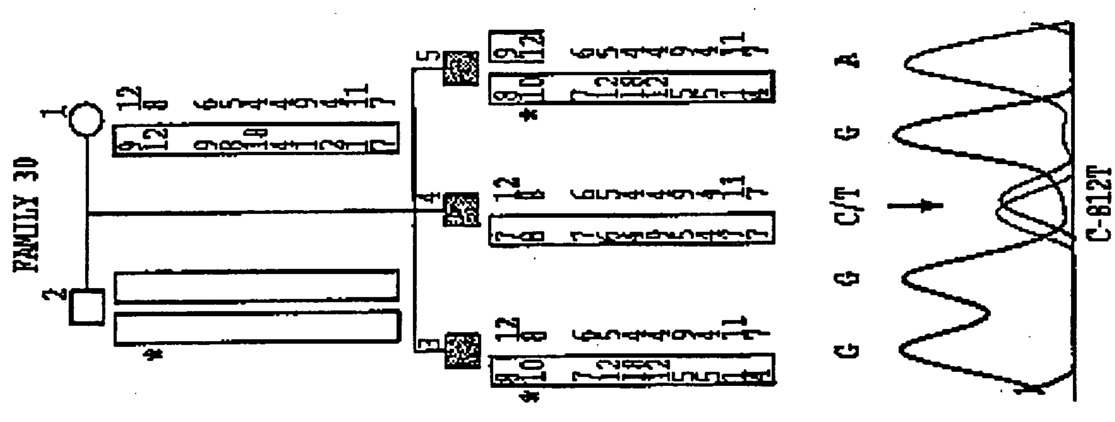


FIG. 2E

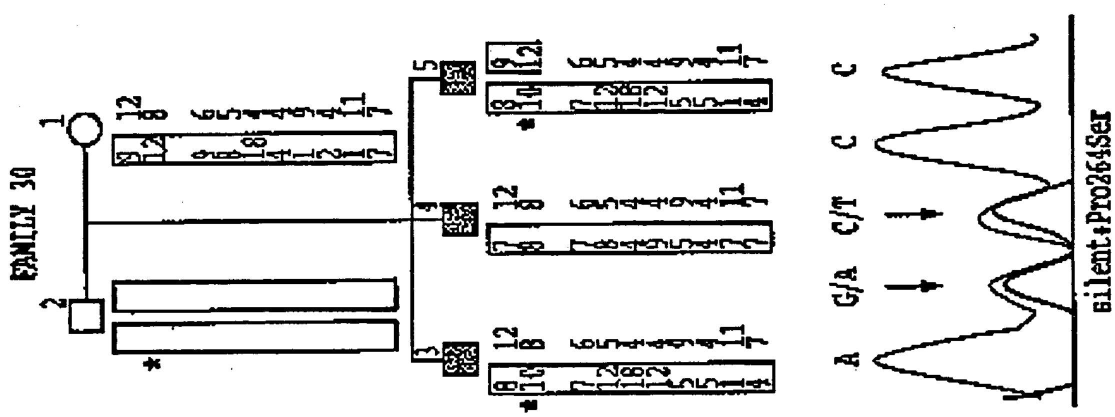


FIG. 2D

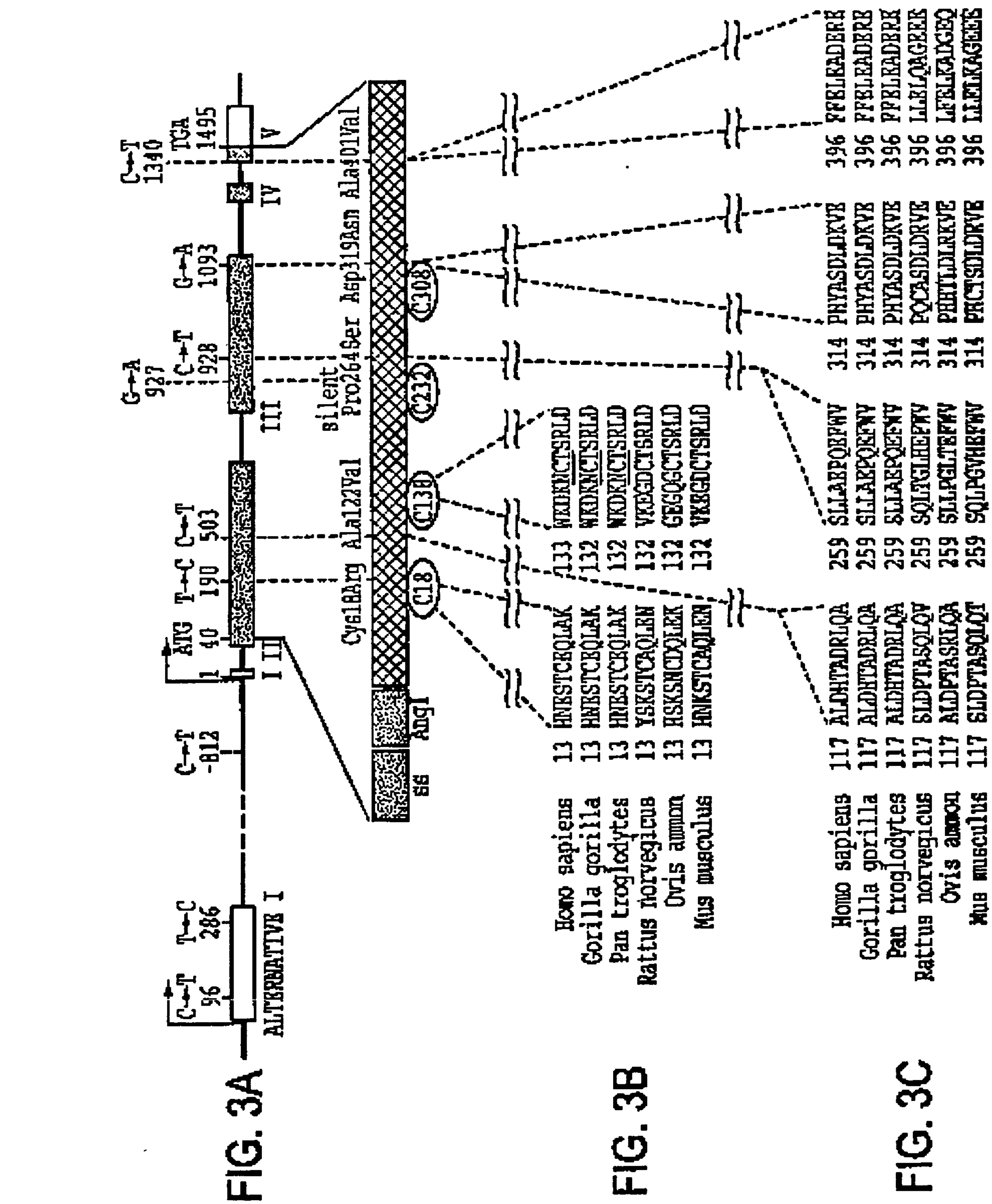


FIG. 3A

FIG. 3B

FIG. 3C

SEQUENCE LISTING

<110> McGrail, Maura
Russell, Deanne L.
Shattuck, Donna M.
Myriad Genetics, Inc.

<120> DIABETES GENE

<130> Diabetes Gene III

<140>
<141>

<150> US 60/174,700
<151> 2000-01-06

<150> US 60/135,423
<151> 1999-05-21

<160> 68

<170> PatentIn Ver. 2.0

<210> 1
<211> 5308
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (3012)..(3047)
<223> exon 1 of AGT gene

<400> 1
gctccagcca cttgacccaa cctggagctc acaaaacccc gtgttgtata aaagtaaggt 60

ttaagggtct agggctgtgt caaagtggct ggggcaaagc tacaaattaa caacatctca 120

gcaaagcaat tgtttaaagt acagctcttt ttcaaaatgg agtctcttat gtcttccctt 180

tctatatgga cacagtaaca gtctgatctc tctttttttt ccctacatat cccaatagca 240

cctgcctgac taatacatca tgctctgctg actccatatg tggctggttt cctggatccg 300

attctgactg atggtatctg ttctcacgct gctaataaag acataccaga gactgggtaa 360

tttataaaga aaaagaggtt taatggactt atgtgttcca cgtggctggg gaggcctcac 420

aatcatggtg gaaggtgaag gaggagcaaa agcacatctt acatggtggc tggcaaaaga 480

gagaaagcat gttcagggga actccccttt ataaaaccat cagatctcat gagacttatg 540

cactatcaca agaacagcac cagaaagatc caccctcatg attcaattac ctcccactgg 600

gtccctcccg tgacacatgg gaattatggg agctacaatt ggagatttgg gtggggacac 660

agccaaacca tatcagatgg cttatttggt ttctatgtag aacctctgct tttcattcaa 720

cagtcttcat ttagccacag atgagctctg tccctaactt ccgctgatgg aatgtacaca 780

taagaaactt ccactgatgg aatgaacacc gaaggtgcct actgggaaga aaactggcct 840
gaatctgagc tgggtcaaat gtctgcagtc agtttgaatg gctgctcctt atgggaataa 900
tttacattct caataaaatt ctctagcaat tttctgattg attttaatga gctttaaagc 960
cttacgtaga agatccccca gctgatagtc agccttgggc atggattaag ggctttaaac 1020
caatcttgca acaagtttaa gcagacattc tttattgggt ccaatctaac caaaattatt 1080
tttttatgtt ctccccagta acgtgtcatt attaagagaa gtttggcttg cttagaggcc 1140
aaatttagag ggtcctgaaa ttttattttc ttttacacca ctttccagca tgttacctga 1200
tcagttgttt attatctttg ctgttgaatg gagtgatcat tccaagggcc cgaggcagga 1260
ggcccaggca cagtggaaac tctcccaaag accaggatct ttgttttgtt ccctgacata 1320
tgctgagcac caggaatagt gcatgaatga aacaaattgt gaggctttaa agagccgaaa 1380
tatttaaaca ctgggcacaa ggttgttgct taatcagtgc tagatcctta cctcccctt 1440
gtgtccaggt cgacttgtta ctgcagttaa accacttgct gatcctcaaa caactagtta 1500
gtggcacagc caggcctagg accccagtct ctactgttcc aactaaccca ttcacaggca 1560
ggagcacttt gaatggtctc ttatttaaaa aaaattaaat taaaattgtc tatttattta 1620
gagacagagt cttactctgt agcccaggct ggaatgcagt ggtgcaatca tagctcactg 1680
taacctccat ctcctggcct caaaaagtgt ttgaattaca gatgcgaggc actgtacctg 1740
gcccgaatgt tctgttcaga caaagccacc tctaagtcgc tgtggggccc cagacaagtg 1800
attttgaggg agtccctatc tataggaaca aagtaattaa aaaaatgtat ttcagaattt 1860
acaggcccat gtgagatatg atttttttaa atgaagattt agagtaatgg gtaaaaaaga 1920
ggtatttgtg tgtttgttga ttgttcagtc agtgaatgta cagcttctgc ctcatatcca 1980
ggcaccatct cttcctgctc tttgttgtta aatgttccat tcctgggtaa tttcatgtct 2040
gccatcgtgg atatgccgtg gctccttgaa cctgcttgtg ttgaagcagg atcttccttc 2100
ctgtcccttc agtgccctaa taccatgtat ttaaggctgg acacatcacc actcccaacc 2160
tgcctcaccc actgcgtcac ttgtgatcac tggcttctgg cgactctcac caaggtctct 2220
gtcatgccct gttataacga ctacaaaagc aagtcttacc tataggaaaa taagaattat 2280
aacccttta ctggtcatgt gaaacttacc atttgcaatt tgtacagcat aaacacagaa 2340
cagcacatct ttcaatgcct gcatcctgaa ggcattttgt ttgtgtcttt caatctggct 2400
gtgctattgt tggtgtttaa cagtctcccc agctacactg gaaacttcca gaaggcactt 2460
ttcacttgct tgtgtgtttt ccccagtgtc tattagaggc ctttgcacag ggtaggctct 2520
ttggagcagc tgaaggtcac acatcccatg agcgggcagc agggtcagaa gtggcccccg 2580

tgttgcctaa gcaagactct cccctgccct ctgccctctg cacctccggc ctgcatgtcc 2640
ctgtggcctc ctggggtaac atctcccggg gctgggtcag aaggcctggg tggttggcct 2700
caggctgtca cacacctagg gagatgctcc cgtttctggg aaccttggcc ccgactcctg 2760
caaacttcgg taaatgtgta actcgaccct gcaccggctc actctgttca gcagtgaaac 2820
tctgcatcga tcactaagac ttcctgggag agggtcccag gtgagtgtcg cttctggcat 2880
ctgtccttct ggccagcctg tggtctggcc aagtgatgta accctcctct ccagcctgtg 2940
cacaggcagc ctgggaacag ctccatcccc acccctcagc tataaatagg gcatcgtgac 3000
ccggccaggg gaagaagctg ccgttgttct gggtcctcca gcaggaggta agcccggggc 3060
cccctcagct ccttctcggc cttgtctctc tcagatgtaa ccgagctgtg ggctaggagg 3120
aaaaggaccg ggaggaggca cggtgatgac tgaaaaacct ctcccctctc ataagaccag 3180
tcatccggac gcgggctttc ccccactcgg tgcccacctg gggtcttaca ggaggagctg 3240
ctcctcctca gcaatgggac aagatggtca ggtcttcctg ttccgctgag aaaagttagg 3300
gtcctcagga acggagcaga ctggtacagg aacagagtca tcatggccaa gagtccaccg 3360
ggtcctcttg ccatcaggag gaatagcagg gcttgtgcag gaattggggc tggagggaag 3420
ggccgggctc ggtcagtctc cagctgggat ccccagagtg gtcaccctac ccctccctcg 3480
agacagactg cctgactgtg tgtcatcagg ctggtcaccg tctccctgaa cctcgatttg 3540
ctcacctata aaatggaact aataacgatg cctgggctcc ctgtctcagg ggctctggta 3600
tagctgaaga gaactaatat aacatgaaag tgctttctaa gctttgggat aagctaaaag 3660
gcagattcca attttattcg agggcaacgt agattggtgc ttcagctcgt ggatgacaga 3720
gtcagggggc ctggttctga gtcctagttc tgtctcttcc cagctgtgtg acgttgaaca 3780
agcccctgga cctctctgtt cctctgcaaa acagcatgaa ccaattcatt aactacttct 3840
ccaggatgca gtaggtccca gggactatcc taggaatgtg ggctgtatta gtaaacacaa 3900
cagcgggaac cctgttccgg ggctcacatt cacatcagag caaacagaca aagacgctgg 3960
acagaataag tgcataacta catggtacag agggttataa ggagggaaaa ggggagctgg 4020
atgagagagt tgagagtgcc cggtgtggtg gggaaagctg cagggtgaaa tactgcatca 4080
gggaaacctc agggaaggtg aggactatgg tgaggtcaga ggggttgata tgagaacagt 4140
gcccgtgcaa tggcaggcac cacaggagca tgagccgtca tcttcacctt tagcattcag 4200
cccgggagaa gtagggagac atagaagggg caggtgctgg ccaagaggca ggggcaggag 4260
aggagaaggc ggaggggcac tcagggcgag ggtgtcaggc ccgccacccc agagcaccat 4320
tactcccagg acgcggctgc gtgcagacct ggaaccagcc taggaagcag ccgcagatca 4380

caactgagaa caaacgacag tctcagcctc aaaaatggcc catggaattg cgtctctgga 4440

gacgctgcct gagcaggagc agcacagtga gcggctgcca ttgaccagcg ccatccaaac 4500

cccgaacagt tggcgcttgt caggcaggac ttcccagcag tcggttccca caggtttccc 4560

ctgttggacc gatttgatgt gactgtctag attaggtgtg aactggtggc ttaggcttct 4620

ctgcacagaa aggcctgcca gcagcagaga gagtttctg ttccattttt ccatgtcatg 4680

tggctcttcc tgagaacagc ggatggagtc aaatgcatgg ggagtggggt gagatggtag 4740

ctgaggtcag aatttggcat ttgaatgact gaagcagaac aaaacacacc cagtacttca 4800

gcagctgcac cgtgttgagg gcaggtgctg gttacgggtc tgggtgaggg aagccagctg 4860

cccatgtaag aagaatgact gggtatgctt agatgaagca gaaaaatcta ggcatcaagg 4920

tggccttgag tcagtgatga cacgctacag ctccaaggaa gcctggccta gccctggggg 4980

gacagaaaag gccaagaagt gacgatattg cagtacagcc ccctccacaa gaaatgagtg 5040

agatgtggta caaaatgtta gaattgaatg aatcaataga ataaacgttc atcccttcaa 5100

tcaagaagag tcagatgaaa tgaattagca gggccagccc aagaacctct tctgggggtc 5160

tcagggtagc tttcatttgt agcagctgag gcggaaccag ctgtaaggcc tttgagagaa 5220

cgtggtgctg gacccgtgtc tagggcaggg gttctaaacc ctgcttacat atcagagtca 5280

cctgagaatc ttcatttttt ttttttt 5308

<210> 2
<211> 1159
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(1009)
<223> exon 2 of AGT gene

<400> 2
aatccatttc tttctttttt tgtagacatg aatggattta ttctgggcta aatggtgaca 60

gggaatattg agacaatgaa agatctggtt agatggcact taaaggtcag ttaataacca 120

cctttcaccc tttgcaaaat gatatttcag ggtatgcgga agcgagcagc ccagtctgag 180

atggctcctg ccggtgtgag cctgaggggc accatcctct gcctcctggc ctgggctggc 240

ctggctgcag gtgaccgggt gtacatacac cccttccacc tcgtcatcca caatgagagt 300

acctgtgagc agctggcaaa ggccaatgcc gggaagccca aagaccccac cttcatacct 360

gctccaattc aggccaagac atcccctgtg gatgaaaagg ccctacagga ccagctggtg 420

ctagtcgctg caaaacttga cacccaagac aagttgaagg ccgcaatggt cgggatgctg 480

gccaacttct tgggcttccg tatatatggc atgcacagtg agctatgggg cgtggtccct 540

ggggccaccg tcctctccca aacggctgtc tttggcaccc tggcctctct ctatctggga 600

gccttggacc acacagctga caggctcaag gcaatcctgg gtgttcactg gaaggacaag 660

aactgcacct cccgggctga tgcgcacaag gtcctgtctg ccctgcaggc tgtacagggc 720

ctgctagtgg cccagggcag ggctgatagc caggcccagc tgctgctgtc cacggtggtg 780

ggcgtgttca cagccccagg cctgcacctg aagcagccgt ttgtgcaggg cctggctctc 840

tatacccctg tggtcctccc acgctctctg gacttcacag aactggatgt tgctgctgag 900

aagattgaca ggttcatgca ggctgtgaca ggatggaaga ctggctgctc cctgacggga 960

gccagtgtgg acagcaccct ggctttcaac acctacgtcc acttccaagg taaggcaaac 1020

ctctctgctg gctctggccc taggacttag tatccaatgt gtagctgaga tcagccagtc 1080

aggccttgga gatgggcagg gggcagccct gcggacatac ctggtgacca cccttgagaa 1140

gtggggaagt ggcctctcc 1159

<210> 3
<211> 568
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(418)
<223> exon 3 of ACT gene

<400> 3
ccaggagaat gcagagtggc ctgggggggg ccaagggaag agggctgagg agggcgaggg 60

gcaggatctg ggcctttggt gtctgccagc cctcattcct gcccctgtct tgggtgactc 120

ttccctccct gtctcctgtc tggatttcag ggaagatgaa gggcttctcc ctgctggccg 180

agccccagga gttctgggtg gacaacagca cctcagtgtc tgttcccatg ctctctggca 240

tgggcacctt ccagcactgg agtgacatcc aggacaactt ctcggtgact caagtgccct 300

tcactgagag cgcctgcctg ctgctgatcc agcctcacta tgcctctgac ctggacaagg 360

tggagggtct cactttccag caaaactccc tcaactggat gaagaaactg tctccccggt 420

aggagcctcc cagtctcccc tggaatgtgg gagccacact gtccctgccc ggctgggggc 480

ggggtgggga gtagacacac ctgagctgag ccttgggtgc agagcagggc agggccgcgg 540

tggcacgggg ctgggcaggc ggcctgtg 568

<210> 4
<211> 445

<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(295)
<223> exon 4 of AGT gene

<400> 4
cccaaggggg aggatgactt agctgggtga tggggggagc aaacatccct gtcgtcaggg 60

ttgggtggaa ggaggaatag cctgcctggc ctctgggaga gccctcactg tgtggcctgg 120

agccttccta acagtgcatc atctccccag gaccatccac ctggccatgc cccaactggt 180

gctgcaagga tctttatgacc tgcaggacct gctcgcccag gctgagctgc ccgccattct 240

gcacaccgag ctgaacctgc aaaaattgag caatgaccgc atcaggggtgg ggggaggtatg 300

tgtgagcctg tgtctgtgcc tgacctgggt tccaagtgtg cacagggtgg gaggcatgga 360

tgtaaggagc acaggggagg ctatgggtgg ggccagcagg gcaagaggga gcggagagta 420

gggccaaagg tgggagagaa gtagc 445

<210> 5
<211> 1091
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(941)
<223> exon 5 of AGT gene

<400> 5
actgtgcatc ctcctggcag gctggaaaat gtccccctcc aggacagtgc acagcacaga 60

ggtcctgagc ccaccccggc cctctagccc tcagcacccct gggtcaccca gtgcgccctc 120

agaatgatcc tgatgtctgc tgctttgcag gtgctgaaca gcatttttttt tgagcttgaa 180

gcggatgaga gagagcccac agagtctacc caacagctta acaagcctga ggtcttggag 240

gtgaccctga accgcccatt cctgtttgct gtgtatgatc aaagcgccac tgccctgcac 300

ttcctgggcc gcgtggccaa ccgctgagc acagcatgag gccagggccc cagaacacag 360

tgcctggcaa ggcctctgcc cctggccttt gaggcaaagg ccagcagcag ataacaaccc 420

cggacaaatc agcgatgtgt caccccccagt ctcccacctt ttcttctaat gagtcgactt 480

tgagctggaa agcagccgtt tctccttggt ctaagtgtgc tgcatggagt gagcagtaga 540

agcctgcagc ggcacaaatg cacctcccag tttgctgggt ttattttaga gaatgggggt 600

ggggaggcaa gaaccagtgt ttagcgcggg actactgttc caaaaagaat tccaaccgac 660

cagcttgttt gtgaaacaaa aaagtgttcc cttttcaagt tgagaacaaa aattgggttt 720

taaaattaaa gtatacattt ttgcattgcc ttcggtttgt atttagtgtc ttgaatgtaa 780

gaacatgacc tccgtgtagt gtctgtaata ccttagtttt ttccacagat gcttgtgatt 840

tttgaacaat acgtgaaaga tgcaagcacc tgaatttctg tttgaatgcg gaaccatagc 900

tggttatttc tcccttgtgt tagtaataaa cgtcttgcaa caataagcat ccaaaaattt 960

tatctttcat ttagcagcca aacagatgta tacaattcag cagatagact gtgcaaacga 1020

aagtgctttc ctggactttg gatggaattt ccatggaagg tctgaggcag tacttagcag 1080

tcctttgaag t 1091

<210> 6
<211> 2099
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (40)..(1494)

<220>
<221> sig_peptide
<222> (40)..(138)

<400> 6
aagaagctgc cgttgttttg ggtactacag cagaagggt atg cgg aag cga gca 54
Met Arg Lys Arg Ala
1 5

ccc cag tct gag atg gct cct gcc ggt gtg agc ctg agg gcc acc atc 102
Pro Gln Ser Glu Met Ala Pro Ala Gly Val Ser Leu Arg Ala Thr Ile
10 15 20

ctc tgc ctc ctg gcc tgg gct ggc ctg gct gca ggt gac cgg gtg tac 150
Leu Cys Leu Leu Ala Trp Ala Gly Leu Ala Ala Gly Asp Arg Val Tyr
25 30 35

ata cac ccc ttc cac ctc gtc atc cac aat gag agt acc tgt gag cag 198
Ile His Pro Phe His Leu Val Ile His Asn Glu Ser Thr Cys Glu Gln
40 45 50

ctg gca aag gcc aat gcc ggg aag ccc aaa gac ccc acc ttc ata cct 246
Leu Ala Lys Ala Asn Ala Gly Lys Pro Lys Asp Pro Thr Phe Ile Pro
55 60 65

gct cca att cag gcc aag aca tcc cct gtg gat gaa aag gcc cta cag 294
Ala Pro Ile Gln Ala Lys Thr Ser Pro Val Asp Glu Lys Ala Leu Gln
70 75 80 85

gac cag ctg gtg cta gtc gct gca aaa ctt gac acc gaa gac aag ttg 342
Asp Gln Leu Val Leu Val Ala Ala Lys Leu Asp Thr Glu Asp Lys Leu
90 95 100

aag gcc gca atg gtc ggg atg ctg gcc aac ttc ttg ggc ttc cgt ata 390
Lys Ala Ala Met Val Gly Met Leu Ala Asn Phe Leu Gly Phe Arg Ile
105 110 115

```
tat ggc atg cac agt gag cta tgg ggc gtg gtc cat ggg gcc acc gtc     438
Tyr Gly Met His Ser Glu Leu Trp Gly Val Val His Gly Ala Thr Val
        120                 125                 130

ctc tcc cca acg gct gtc ttt ggc acc ctg gcc tct ctc tat ctg gga     486
Leu Ser Pro Thr Ala Val Phe Gly Thr Leu Ala Ser Leu Tyr Leu Gly
    135                 140                 145

gcc ttg gac cac aca gct gac agg cta cag gca atc ctg ggt gtt cct     534
Ala Leu Asp His Thr Ala Asp Arg Leu Gln Ala Ile Leu Gly Val Pro
150                 155                 160                 165

tgg aag gac aag aac tgc acc tcc cgg ctg gat gcg cac aag gtc ctg     582
Trp Lys Asp Lys Asn Cys Thr Ser Arg Leu Asp Ala His Lys Val Leu
                170                 175                 180

tct gcc ctg cag gct gta cag ggc ctg cta gtg gcc cag ggc agg gct     630
Ser Ala Leu Gln Ala Val Gln Gly Leu Leu Val Ala Gln Gly Arg Ala
            185                 190                 195

gat agc cag gcc cag ctg ctg ctg tcc acg gtg gtg ggc gtg ttc aca     678
Asp Ser Gln Ala Gln Leu Leu Leu Ser Thr Val Val Gly Val Phe Thr
        200                 205                 210

gcc cca ggc ctg cac ctg aag cag ccg ttt gtg cag ggc ctg gct ctc     726
Ala Pro Gly Leu His Leu Lys Gln Pro Phe Val Gln Gly Leu Ala Leu
    215                 220                 225

tat acc cct gtg gtc ctc cca cgc tct ctg gac ttc aca gaa ctg gat     774
Tyr Thr Pro Val Val Leu Pro Arg Ser Leu Asp Phe Thr Glu Leu Asp
230                 235                 240                 245

gtt gct gct gag aag att gac agg ttc atg cag gct gtg aca gga tgg     822
Val Ala Ala Glu Lys Ile Asp Arg Phe Met Gln Ala Val Thr Gly Trp
                250                 255                 260

aag act ggc tgc tcc ctg acg gga gcc agt gtg gac agc acc ctg gct     870
Lys Thr Gly Cys Ser Leu Thr Gly Ala Ser Val Asp Ser Thr Leu Ala
            265                 270                 275

ttc aac acc tac gtc cac ttc caa ggg aag atg aag ggc ttc tcc ctg     918
Phe Asn Thr Tyr Val His Phe Gln Gly Lys Met Lys Gly Phe Ser Leu
        280                 285                 290

ctg gcc gag ccc cag gag ttc tgg gtg gac aac agc acc tca gtg tct     966
Leu Ala Glu Pro Gln Glu Phe Trp Val Asp Asn Ser Thr Ser Val Ser
    295                 300                 305

gtt ccc atg ctc tct ggc atg ggc acc ttc cag cac tgg agt gac atc     1014
Val Pro Met Leu Ser Gly Met Gly Thr Phe Gln His Trp Ser Asp Ile
310                 315                 320                 325

cag gac aac ttc tcg gtg act caa gtg ccc ttc act gag agc gcc tgc     1062
Gln Asp Asn Phe Ser Val Thr Gln Val Pro Phe Thr Glu Ser Ala Cys
                330                 335                 340

ctg ctg ctg atc cag cct cac tat gcc tct gac ctg gac aag gtg gag     1110
Leu Leu Leu Ile Gln Pro His Tyr Ala Ser Asp Leu Asp Lys Val Glu
            345                 350                 355
```

ggt ctc act ttc cag caa aac tcc ctc aac tgg atg aag aaa ctg tct 1158
Gly Leu Thr Phe Gln Gln Asn Ser Leu Asn Trp Met Lys Lys Leu Ser
360 365 370

ccc cgg acc atc cac ctg acc atg ccc caa ctg gtg ctg caa gga tct 1206
Pro Arg Thr Ile His Leu Thr Met Pro Gln Leu Val Leu Gln Gly Ser
375 380 385

tat gac ctg cag gac ctg ctc gcc cag gct gag ctg ccc gcc att ctg 1254
Tyr Asp Leu Gln Asp Leu Leu Ala Gln Ala Glu Leu Pro Ala Ile Leu
390 395 400 405

cac acc gag ctg aac ctg caa aaa ttg agc aat gac cgc atc agg gtg 1302
His Thr Glu Leu Asn Leu Gln Lys Leu Ser Asn Asp Arg Ile Arg Val
410 415 420

ggg gag gtg ctg aac agc att ttt ttt gag ctt gaa gcg gat gag agg 1350
Gly Glu Val Leu Asn Ser Ile Phe Phe Glu Leu Glu Ala Asp Glu Arg
425 430 435

gag ccc aca gag tct acc caa cag ctt aac aag cct gag gtc ttg gag 1398
Glu Pro Thr Glu Ser Thr Gln Gln Leu Asn Lys Pro Glu Val Leu Glu
440 445 450

gtg acc ctg aac cgc cca ttc ctg ttt gct gtg tat gat caa agc gcc 1446
Val Thr Leu Asn Arg Pro Phe Leu Phe Ala Val Tyr Asp Gln Ser Ala
455 460 465

act gcc ctg cac ttc ctg ggc cgc gtg gcc aac ccg ctg agc aca gca 1494
Thr Ala Leu His Phe Leu Gly Arg Val Ala Asn Pro Leu Ser Thr Ala
470 475 480 485

tgaggccagg gccccagaac acagtgcctg gcaaggcctc tgcccctggc ctttgaggca 1554

aaggccagca gcagataaca accccggaca aatcagcgat gtgtcacccc cagtctccca 1614

ccttttcttc taatgagtcg actttgagct ggaaagcagc cgtttctcct tggtctaagt 1674

gtgctgcatg gagtgagcag tagaagcctg cagcggcaca aatgcacctc ccagtttgct 1734

gggtttattt tagagaatgg gggtggggag gcaagaacca gtgttttagc gcgggactct 1794

gttccaaaaa gaatcccaac cgaccagctt gtttgtgaaa caaaaaagtg ttcccttttc 1854

aagttgagaa caaaaattgg gttttaaaat taaagtatac atttttgcat tgccttcggt 1914

ttgtatttag tgtcttgaat gtaagaacat gacctccgtg tagtgtctgt aatacattag 1974

ttttttccac agatgcttgt gatttttgaa caatacgtga aagatgcaag cacctgaatt 2034

tctgtttgaa tgcggaacca tagctggtta tttctccctt gtgttagtaa taaacgtctt 2094

gccac 2099

<210> 7
<211> 485
<212> PRT
<213> Homo sapiens

```
<400> 7
Met Arg Lys Arg Ala Pro Gln Ser Glu Met Ala Pro Ala Gly Val Ser
 1               5                  10                  15

Leu Arg Ala Thr Ile Leu Cys Leu Leu Ala Trp Ala Gly Leu Ala Ala
            20                  25                  30

Gly Asp Arg Val Tyr Ile His Pro Phe His Leu Val Ile His Asn Glu
        35                  40                  45

Ser Thr Cys Glu Gln Leu Ala Lys Ala Asn Ala Gly Lys Pro Lys Asp
    50                  55                  60

Pro Thr Phe Ile Pro Ala Pro Ile Gln Ala Lys Thr Ser Pro Val Asp
65                  70                  75                  80

Glu Lys Ala Leu Gln Asp Gln Leu Val Leu Val Ala Ala Lys Leu Asp
                85                  90                  95

Thr Glu Asp Lys Leu Lys Ala Ala Met Val Gly Met Leu Ala Asn Phe
            100                 105                 110

Leu Gly Phe Arg Ile Tyr Gly Met His Ser Glu Leu Trp Gly Val Val
        115                 120                 125

His Gly Ala Thr Val Leu Ser Pro Thr Ala Val Phe Gly Thr Leu Ala
    130                 135                 140

Ser Leu Tyr Leu Gly Ala Leu Asp His Thr Ala Asp Arg Leu Gln Ala
145                 150                 155                 160

Ile Leu Gly Val Pro Trp Lys Asp Lys Asn Cys Thr Ser Arg Leu Asp
                165                 170                 175

Ala His Lys Val Leu Ser Ala Leu Gln Ala Val Gln Gly Leu Leu Val
            180                 185                 190

Ala Gln Gly Arg Ala Asp Ser Gln Ala Gln Leu Leu Leu Ser Thr Val
        195                 200                 205

Val Gly Val Phe Thr Ala Pro Gly Leu His Leu Lys Gln Pro Phe Val
    210                 215                 220

Gln Gly Leu Ala Leu Tyr Thr Pro Val Val Leu Pro Arg Ser Leu Asp
225                 230                 235                 240

Phe Thr Gln Leu Asp Val Ala Ala Glu Lys Ile Asp Arg Phe Met Gln
                245                 250                 255

Ala Val Thr Gly Trp Lys Thr Gly Cys Ser Leu Thr Gly Ala Ser Val
            260                 265                 270

Asp Ser Thr Leu Ala Phe Asn Thr Tyr Val His Phe Gln Gly Lys Met
        275                 280                 285

Lys Gly Phe Ser Leu Leu Ala Glu Pro Gln Glu Phe Trp Val Asp Asn
    290                 295                 300

Ser Thr Ser Val Ser Val Pro Met Leu Ser Gly Met Gly Thr Phe Gln
305                 310                 315                 320
```

```
His Trp Ser Asp Ile Gln Asp Asn Phe Ser Val Thr Gln Val Pro Phe
                325                 330                 335

Thr Glu Ser Ala Cys Leu Leu Leu Ile Gln Pro His Tyr Ala Ser Asp
            340                 345                 350

Leu Asp Lys Val Glu Gly Leu Thr Phe Gln Gln Asn Ser Leu Asn Trp
        355                 360                 365

Met Lys Lys Leu Ser Pro Arg Thr Ile His Leu Thr Met Pro Gln Leu
    370                 375                 380

Val Leu Gln Gly Ser Tyr Asp Leu Glu Asp Leu Leu Ala Gln Ala Glu
385                 390                 395                 400

Leu Pro Ala Ile Leu His Thr Glu Leu Asn Leu Gln Lys Leu Ser Asn
                405                 410                 415

Asp Arg Ile Arg Val Gly Glu Val Leu Asn Ser Ile Phe Phe Glu Leu
            420                 425                 430

Glu Ala Asp Glu Arg Glu Pro Thr Glu Ser Thr Gln Gln Leu Asn Lys
        435                 440                 445

Pro Glu Val Leu Glu Val Thr Leu Asn Arg Pro Phe Leu Phe Ala Val
    450                 455                 460

Tyr Asp Glu Ser Ala Thr Ala Leu His Phe Leu Gly Arg Val Ala Asn
465                 470                 475                 480

Pro Leu Ser Thr Ala
                485
```

<210> 8
<211> 681
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (151)..(531)
<223> altenate exon 1 of AGT gene

<400> 8

```
agggcgggga aggggggagg aagggggact gagactctcc tgtttctata tgctatgatg 60

ggatttgacc tgcaattgaa cctttcctca aacacaaggc acaaacctgc cccagcccat 120

catcctccca cagaggcact gtgaatgtat aaagaaaggc gatgctaaga aacgtgctgc 180

aggggaggct ggcacattct gctccttagc taaccgcaca ggcccatggg tccacacaca 240

tcagaccatg gctctctgtg gtctatcttt gccattactt taaccaggct gtttctcatg 300

cgctcatgga gggtgactga agatggcacc ccgaggtgca ccctcagcct cgctggacac 360

ttacggctcg cattctccag ccggaccagg cagttggagg agtcatcgaa gtccagctgg 420
```

agctcctcat ccgcatacct gagcacaatc agctgcagga ggtggctgct cagctgaaag 480

cctgtgggga caaaggagga gacccaggcc tcgctgtatc cgagccccga ggtgagtctg 540

caccttgcag ggagctccca ggcagctgag gagagggctc agcagcagca gccactgttt 600

cactcctcaa agctggggtc cccagaccca gtgggactgc tgcccttggt gtgaatgcag 660

cgtgagagtg tgaatctgtg g 681

<210> 9
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:M13 forward
primer

<400> 9
gttttcccag tcacgacg 18

<210> 10
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:M13 reverse
primer

<400> 10
aggaaacagc tatgaccat 19

<210> 11
<211> 20
<212> DNA
<213> Homo sapiens

<400> 11
cctcgaagtg gcctatggaa 20

<210> 12
<211> 20
<212> DNA
<213> Homo sapiens

<400> 12
gcgagactct atgggtgatg 20

<210> 13
<211> 20
<212> DNA
<213> Homo sapiens

<400> 13
gactgagact ctcctgtttc 20

<210> 14
<211> 19
<212> DNA
<213> Homo sapiens

<400> 14
gggggtggca tcttcagtc 19

<210> 15
<211> 20
<212> DNA
<213> Homo sapiens

<400> 15
tggcacattc tgctccttag 20

<210> 16
<211> 20
<212> DNA
<213> Homo sapiens

<400> 16
atacagcgag tcctgggtct 20

<210> 17
<211> 20
<212> DNA
<213> Homo sapiens

<400> 17
gaccaggcag ttgaggaagt 20

<210> 18
<211> 20
<212> DNA
<213> Homo sapiens

<400> 18
gattcacact ctcacgctgc 20

<210> 19
<211> 20
<212> DNA
<213> Homo sapiens

<400> 19
ccactttcca gcatgttacc 20

<210> 20
<211> 20

<212> DNA
<213> Homo sapiens

<400> 20
ctgctattcc tcctgatggc 20

<210> 21
<211> 20
<212> DNA
<213> Homo sapiens

<400> 21
acaggcccat gtgagatatg 20

<210> 22
<211> 20
<212> DNA
<213> Homo sapiens

<400> 22
tggtattagg gcactgaagg 20

<210> 23
<211> 20
<212> DNA
<213> Homo sapiens

<400> 23
tgtgttgaag caggatcttc 20

<210> 24
<211> 20
<212> DNA
<213> Homo sapiens

<400> 24
gatgcaggca ttgaaagatg 20

<210> 25
<211> 21
<212> DNA
<213> Homo sapiens

<400> 25
gtgtttaaca gtctccccag c 21

<210> 26
<211> 20
<212> DNA
<213> Homo sapiens

<400> 26
ctaggtgtgt gacagcctga 20

<210> 27
<211> 19
<212> DNA
<213> Homo sapiens

<400> 27
catgtccctg tggcctctt 19

<210> 28
<211> 18
<212> DNA
<213> Homo sapiens

<400> 28
tgaggggtgg ggatggag 18

<210> 29
<211> 20
<212> DNA
<213> Homo sapiens

<400> 29
ctggtcatgt gaaacttacc 20

<210> 30
<211> 20
<212> DNA
<213> Homo sapiens

<400> 30
accttcagct gctccaaaga 20

<210> 31
<211> 20
<212> DNA
<213> Homo sapiens

<400> 31
ccacttccca gcatgttacc 20

<210> 32
<211> 20
<212> DNA
<213> Homo sapiens

<400> 32
ctgctattcc tcctgatggc 20

<210> 33
<211> 18
<212> DNA
<213> Homo sapiens

<400> 33
aaccctcctc tccagcct 18

<210> 34
<211> 18
<212> DNA
<213> Homo sapiens

<400> 34
tcatcaaagt gcctcctc 18

<210> 35
<211> 20
<212> DNA
<213> Homo sapiens

<400> 35
tacttggact ttgggctgag 20

<210> 36
<211> 21
<212> DNA
<213> Homo sapiens

<400> 36
ttgagcaagg cactttgttt c 21

<210> 37
<211> 21
<212> DNA
<213> Homo sapiens

<400> 37
ggctaaatgg tgacagggaa t 21

<210> 38
<211> 20
<212> DNA
<213> Homo sapiens

<400> 38
tagggccttt tcatccacag 20

<210> 39
<211> 20
<212> DNA
<213> Homo sapiens

<400> 39
aatgccggga agcccaaaga 20

<210> 40
<211> 20

<212> DNA
<213> Homo sapiens

<400> 40
tccaaggctc ccagatagag 20

<210> 41
<211> 20
<212> DNA
<213> Homo sapiens

<400> 41
ctctccccaa cggctgtctt 20

<210> 42
<211> 20
<212> DNA
<213> Homo sapiens

<400> 42
ctgcacaaac ggctgcttca 20

<210> 43
<211> 20
<212> DNA
<213> Homo sapiens

<400> 43
ctctatctgg gagccttgga 20

<210> 44
<211> 20
<212> DNA
<213> Homo sapiens

<400> 44
cacagcctgc atgaacctgt 20

<210> 45
<211> 20
<212> DNA
<213> Homo sapiens

<400> 45
tgaagcagcc gtttgtgcag 20

<210> 46
<211> 19
<212> DNA
<213> Homo sapiens

<400> 46
tccccacttc tcagaggtg 19

<210> 47
<211> 20
<212> DNA
<213> Homo sapiens

<400> 47
acaggttcat gcaggctgtg 20

<210> 48
<211> 19
<212> DNA
<213> Homo sapiens

<400> 48
tccccacttc tcaagggtg 19

<210> 49
<211> 21
<212> DNA
<213> Homo sapiens

<400> 49
tgcctcagtg aactcaaatg g 21

<210> 50
<211> 20
<212> DNA
<213> Homo sapiens

<400> 50
ctccccctcc aaaactacca 20

<210> 51
<211> 19
<212> DNA
<213> Homo sapiens

<400> 51
caggagaatg cagagtggc 19

<210> 52
<211> 19
<212> DNA
<213> Homo sapiens

<400> 52
gcaggcgctc tcagtgaag 19

<210> 53
<211> 20
<212> DNA
<213> Homo sapiens

<400> 53
agcactggag tggcatccag 20

<210> 54
<211> 20
<212> DNA
<213> Homo sapiens

<400> 54
gatggaggac tggtagacag 20

<210> 55
<211> 20
<212> DNA
<213> Homo sapiens

<400> 55
aagggggagg atgacttagt 20

<210> 56
<211> 21
<212> DNA
<213> Homo sapiens

<400> 56
gaattccctt ctaaagtcca g 21

<210> 57
<211> 20
<212> DNA
<213> Homo sapiens

<400> 57
gtgcaaggag cataagcctg 20

<210> 58
<211> 19
<212> DNA
<213> Homo sapiens

<400> 58
ctactctccg ctccctctt 19

<210> 59
<211> 20
<212> DNA
<213> Homo sapiens

<400> 59
aagggggagg atgacttagt 20

<210> 60
<211> 21

<212> DNA
<213> Homo sapiens

<400> 60
gaattcccat ccaaagtcca g 21

<210> 61
<211> 17
<212> DNA
<213> Homo sapiens

<400> 61
tagccctcag caccctg 17

<210> 62
<211> 19
<212> DNA
<213> Homo sapiens

<400> 62
tgtcaggggt tgttatctg 19

<210> 63
<211> 20
<212> DNA
<213> Homo sapiens

<400> 63
agaacacagt gcctggcaag 20

<210> 64
<211> 20
<212> DNA
<213> Homo sapiens

<400> 64
ttcacaaaca agctggtcgg 20

<210> 65
<211> 20
<212> DNA
<213> Homo sapiens

<400> 65
agtgtttagc gcgggactac 20

<210> 66
<211> 20
<212> DNA
<213> Homo sapiens

<400> 66
tttggaggct tattgtggca 20

<210> 67
<211> 20
<212> DNA
<213> Homo sapiens

<400> 67
tgaaagatgc uoqcacctga 20

<210> 68
<211> 20
<212> DNA
<213> Homo sapiens

<400> 68
gaaagcactt tcgtttgcac 20

This Page Blank (uspto)